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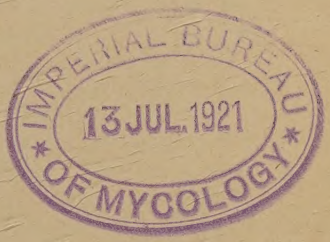
EXPERIMENT STATION



BOTANICAL DEPARTMENT

THE LEAF-SPOT DISEASE OF TOMATO

BY

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EAST LANSING, MICHIGAN
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FOREWORD.

The importance of the tomato crop in the state of Michigan and the seriousness of some of the losses occasioned by diseases led the Department of Botany to begin investigations on the subject several years ago. The investigation of one of these diseases, the leaf-spot, the subject of this bulletin, was turned over to one of the advanced students, Mr. Ezra Levin, who began work in the fall of 1913 and devoted a very large part of the following school year and summer to the problem. The work was carried out along lines laid down by Dr. G. H. Coons, Research Assistant in Plant Pathology, who kept very close supervision of Mr. Levin's work. The revision of the manuscript and the larger part of the preparation of the Bibliography were also carried out by Dr. Coons. The result of the investigations throw great light upon the structure and life history of the fungus (*Septoria lycopersici*) causing the disease and explain many of the apparently contradictory observations and conclusions of previous investigators. They are of especial value as they show where lie the vulnerable points in the life history of the fungus, an item of the utmost importance in the combating of a disease. Furthermore, the facts learned have been found to shed light upon other diseases caused by other species of Septoria, such as the late blight of celery, etc.

In view of the foregoing it is believed that it is highly desirable to publish the details of the investigations as a Technical Bulletin, for the benefit of investigators wherever the diseases of this type are being studied, and to present the main facts, together with directions for control, in a popular form in another publication.

ERNST A. BESSEY,
Botanist.

CONTENTS.

THE LEAF-SPOT DISEASE OF TOMATO.

THE HOST.

The common commercial type of tomato is *Lycopersicon lycopersicon* (L.) Karst., and is to be distinguished from the currant tomato, *Lycopersicon pimpinellifolium* Dunal, and the cherry tomato, *Lycopersicon cerasiforme* Dunal.

The importance of the tomato crop in the United States is indicated by the following statement from the Thirteenth Census Report: "Judged by value, tomatoes were the most important vegetable, the value exceeding $13,700,000 in 1909." The Michigan tomato crop is extremely valuable. The report credits the state with $217,256, tomato production, and this has since increased to at least five times this amount.

The methods used in growing this crop influence the prevalence of this disease to a great extent.

Seed is sown in hot beds or greenhouses several weeks before setting out; (in Michigan from March to May). In the open, two types of culture are employed: (1) training of plants to two or three stems tied to a stake, (2) allowing plants to grow over the ground at will. In forcing tomatoes, the plants are usually trained to a single stem and staked.

In recent years more and more complaints, indicating the prevalence of serious tomato diseases, have come from the tomato growers of Michigan. The plant disease survey of the state indicated that Septoria leaf-spot was especially serious. Investigation was begun by the writer in September, 1913, and practically completed August, 1914.

THE DISEASE.

The common names by which this serious disease is known are: "late blight," "blight," and "leaf-spot." It is, however, a well known fact that in some sections of the country, tomatoes are attacked by *Phytophthora infestans* (Mont.) de By., the cause of late blight of potato. This precludes the use of "late blight" as a name for this disease. "Blight" and "leaf-spot" are also more or less preempted. The former of these terms or preferably "early blight" may best be associated with *Alternaria solani* (E. and M.) J. and G. which also attacks tomatoes. A common usage among pathologists seems to warrant the name of "leaf-spot" in referring to the diseases of this type.

PREVIOUS INVESTIGATION OF THE DISEASE AND ITS CAUSAL ORGANISM.

It is generally conceded that the fungus, *Septoria lycopersici* Speg. causes the disease commonly known as "leaf-spot" of tomato. As will be seen this has been more of an inference based upon the constant asso-

ciation of the organism with the disease than a conclusion resulting from experimentation.

The organism was discovered in Argentina by Spegazzini in 1882. The fungus and its characteristic lesions were tersely described by him in a publication containing descriptions of several other organisms. The name given by Spegazzini has been in common use for this disease-producing organism although the fungus was subsequently found upon the host in countries far from the source of the type material.

The early accounts of this disease consisted of a few scattered notes recording the finding of the fungus in various localities. For example, as recorded in Briosi and Cavara (1889): Cuboni (1888) and Passerini (1889) found the fungus in Italy. Passerini's contribution is noteworthy in that this author recognized the pathogenic character of the organism and outlined rather fully the symptoms of the disease.

B. D. Halsted (1896; 1897) was the first to report the disease for the United States and in one of his annual reports he listed *Septoria lycopersici* for New Jersey and mentioned it as causing one of the leading fungous diseases of tomato. A. D. Selby (1897a) discussed a "new arrival" in Ohio, apparently *Septoria lycopersici,* and in a later article, its distribution in Ohio was particularly noted (1897b). The same author in 1899 stated—"During the season 1898 conditions of warmth and moisture seemed to favor the development of the Septoria fungus." In 1899, Dr. E. A. Bessey observed extensive damage in Maryland near Annapolis Junction (verbal communication). F. S. Earle (1900) noted a serious epidemic of this disease in the cold frames at Auburn, Ala. N. A. Cobb (1902), discussing tomato diseases of Australia, stated that this disease, "has never been mentioned in this journal (Agr. Gaz. N. S. Wales) hitherto." "The leaves are attacked more particularly although there is hardly any part of the plant entirely free from attack." Again recording the fungus for Australia, D. McAlpine (1903) gave a description of the fungus and the effect produced upon the host. W. A. Kellerman (1903) wrote a brief note on *Septoria lycopersici.* In 1904, W. A. Orton mentioned the disease as serious in the previous year. G. Delacroix (1905) in recording the history of this fungus in France, wrote a description of the causal organism on the host and in pure culture and noted successful infection experiments. (See page 12). L. Reh (1905) described the disease in Germany. G. Köck (1905) noted the disease in Austria. Later he described *Septoria lycopersici,* and made a series of observations on its effects on the host, and resistance of varieties. K. Kornauth (1905) gave a further account of the disease in Austria. J. Burtt-Davy (1905) records the disease from Transvaal. J. L. Sheldon, in West Virginia (1905), noted "Leaf-Spot." B. F. Floyd (1905), in Missouri, described the disease as attacking "leaves, stems, and sometimes green fruit." L. Hollos (1907) described the disease in Hungary. F. D. Kern noted *Septoria lycopersici* in 1906 in Indiana. H. T. Güssow (1908) gave a full description of the disease. This article, dealing chiefly with greenhouse conditions, is an extensive discussion of the fungus and the disease which it produces. The symptoms of the disease are discussed in detail, inconclusive inoculation experiments are reported, and various inferences as to the dissemination of the organism are drawn. F. L. Stevens and J. G. Hall (1909) used *Septoria lycopersici* for experi-

ments reported in a paper entitled "Variation of Fungi due to Environment." This article is chiefly devoted to a biometric study of Septoria fruiting bodies but from it more or less of a description of the organism in pure culture can be gleaned. The Great Britain Agriculture and Fisheries Board (1909) again drew attention to the disease in a popular article taken largely from Güssow's. H. W. Barre (1910) noted the disease and its importance in South Carolina. J. W. Lloyd and I. S. Brooks (1910), Illinois, gave a brief description of "Leaf-Spot." H. S. Reed (1911) reported damage done by the organism in Virginia and gave the most complete description of field conditions of the disease up to that time. Long (1913) gave a popular account of the disease under English conditions. J. B. S. Norton (1914), discussing the disease in Maryland, gave the results of spraying experiments, as well as a summary of the previous spraying tests of other stations.

ECONOMIC IMPORTANCE.

That this disease has long been of importance in different parts of the world is apparent from the reports of N. A. Cobb (1902), D. McAlpine (1903), G. Köck (1905), and H. T. Güssow (1908), to which reference has already been made.

In the United States the disease has spread more or less rapidly, particularly within the last few years. The damage resulting from it ranges from three percent to a total loss of the crop. Practically all the states that raise tomatoes to any degree commercially are more or less troubled with leaf-spot, as is shown by the accompanying map (Figure 1) which summarizes data obtained by a questionnaire, addressed to American pathologists.

The following comments taken from the replies are interesting:

J. B. S. Norton, of Maryland, in answer to the questionnaire states: "The disease causes a loss of 10-25 per cent of our tomato crop. The loss will run into hundreds of thousands of dollars, if not a million." For Missouri, Geo. M. Reed writes: "In some fields perhaps 50% of the early tomato crop is damaged by this disease." N. J. Giddings, of West Virginia: "It is the worst disease which we have on tomatoes in West Virginia." C. E. Durst, of Illinois, states: "This disease coupled with Fusarium Wilt is almost driving the growers out of the business of tomato growing in that section (southern Illinois)." C. W. Edgerton, Louisiana, writes: "Not as yet common but seems to be spreading rapidly. I look for this disease to become serious in this state." It is noteworthy that Septoria leaf-spot has evidently appeared in Louisiana but recently. H. S. Reed (1905): "The culture of tomatoes in southwestern Virginia is becoming increasingly difficult, due to the serious blight and point rot diseases, which have attacked this crop annually for the past five years." On the contrary, H. W. Anderson, Wabash College, Indiana, writes: "In this region we consider it a benefit, since it kills off the leaves near the base of the plant and allows the sun to ripen the tomatoes."

DISTRIBUTION.

In answer to a questionnaire as to prevalence, the pathologists of thirty-four states reported the disease as *common* from the following: Ala-

bama, California, Connecticut, Delaware, Illinois, Louisiana (becoming common), Maryland, Michigan, Missouri, New Jersey, New York, North Carolina, Pennsylvania, and Wisconsin. (Figure 1).

A reply to a similar request to the Bureau of Plant Industry, Plant Disease Survey (Cotton and Truck Disease and Sugar Plant Investigations) for information as to the distribution of the disease brought the following: "Reported in 1903 from Alabama, Connecticut, Missouri, New Jersey, New York, and Ohio; 1904 from North Carolina and Ohio; 1905 from Delaware, Maryland, North Carolina, Ohio, and West Virginia; 1906 from Delaware, Nebraska, New Jersey, Ohio, and West Virginia; 1907 from Delaware, Illinois (mentioned that it has been present in this state for three or four years), Maryland, Nebraska, New Jersey, North

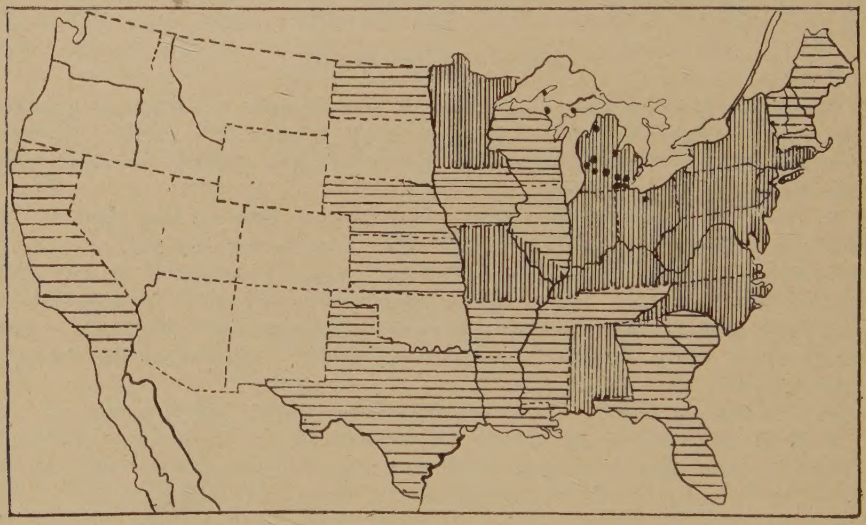

Figure 1. Distribution and relative importance of the Septoria leaf-spot of Tomato, as determined from a questionnaire to American phytopathologists. Collection records from Michigan are shown by dots.

Carolina, Ohio, and West Virginia; 1908 from Delaware, Maryland, New Jersey, Ohio, Pennsylvania, and South Carolina; 1909, Delaware, Indiana, Iowa, Maryland, Nebraska, New Jersey, North Carolina, Ohio, Pennsylvania, South Carolina, and West Virginia; 1910 from Delaware, Indiana, Kansas, Kentucky, (exceptionally prevalent), Maryland, Mississippi, Nebraska, New York, Ohio, South Carolina, Texas, Virginia, and West Virginia; 1911 from Delaware, District of Columbia, Florida, Illinois, Iowa, Maryland, Massachusetts, Missouri, Ohio, Pennsylvania, Virginia, West Virginia and Wisconsin; 1912 from Alabama, Delaware, District of Columbia, Illinois, Indiana, Iowa, Maryland, Michigan, Minnesota, Missouri, New Jersey, North Carolina, Ohio, Rhode Island, South Carolina, Tennessee, Texas, Virginia, and West Virginia." The reply also notes that "The disease may not necessarily have progressed as rapidly as this would indicate but may have been present in all the states earlier."

SIGNS.

The general signs of this tomato disease are conveyed by the popular term, "blight." The growers notice a general unthriftiness in their plants, along with a dying of the leaves beginning first with the lower ones and gradually advancing upward on the plant. (Plate 1). The fruit fails to ripen evenly or rapidly, and with severe attack, the entire crop may fail. Such fruit as is produced from diseased plants, lacks sweetness and solid matter. This last condition is often complained of by canners, although they ascribe "watery" tomatoes to wet season, rather than to a fungous disease made severe by the season. In short, all the general signs which accompany interference with the photosynthetic function are to be expected with this disease. The great reduction in leaf surface is accompanied by a reduction of carbohydrate formation.

Along with these general signs of the disease there are distinct lesions on various parts of the plant which allow sure diagnosis.

The fungus causes lesions upon the leaf, the stem, the fruit and the calyx.

On the Leaves:

The earliest indication of the disease is a water-soaked spot which can be distinguished with a hand lens on the underside of the leaf. There is no noticeable discoloration of the tissue at the outset. As the spot grows larger it becomes more or less circular in outline and shows a definite margin. The affected tisssue darkens, becomes shrunken and later appears hard and dry. The color of this spot may vary from black to grayish-white. The spots may vary in shape and size from a small circular spot of pin-head size to a large irregular spot of about 2 cm. in diameter. Not infrequently the spots coalesce. While the tissue is shrinking 3-10 small, black, glistening pycnidia appear in the spot. Finally, yellowish, mucilaginous masses can be seen exuding from the pycnidia. Upon microscopic examination these are found to be masses of long filiform spores. The number of pycnidia in the larger spots may be as high as 30. The pycnidia are well defined, visible with the naked eye and separate (Plate 2.). Pycnidia may occur on the under side of the leaf; usually, however, they occur on the upper side. At this point it must be noted that not all spots contain pycnidia when the leaf dies. This point will be taken up in detail later. (Page 24).

About the time of spore exudation, the green tissue of the leaflet contiguous to the fungous spot begins to turn yellow. This yellowing increases, eventually involving the entire leaflet. Then the fungous spots which have so far been pliable become dry and brittle. The leaflets gradually droop and dry on the stalk which later also shrivels up but remains attached to the stem until broken off by a slight jar. On tomatoes which are staked the disease is sometimes confined to the lower leaves (Plate 1). Where the plants are allowed to trail at will, the disease may cause almost complete defoliation of the plant, the small tufts of young, terminal leaves alone escaping.

On the Stems:

The disease is commonly found on the stems. It is manifested by small, slightly elongated, dark spots containing pycnidia. These spots

are not so clearly defined as those on the leaf. The damage to the stem is slight; these spots do not enlarge to form cankers and are not serious except insofar as they produce spores for further infection.

On the Calyx:

Small spots, more or less elongated, occur on the calyx and take a form intermediate between those on the leaves and stems. It may be remarked that the differences in form of the spot are doubtless due to the texture of the host.

On the Fruit:

The disease has never been seen in Michigan as affecting the fruit in the field, however, J. B. S. Norton, (1914) states, "The disease may also attack the fruit, causing dark spots." It will be seen later that artificial inoculation with spores does not lead to infection of fruit, unless the epidermis is ruptured. In general it may be noted that the damage to the fruit by leaf-spot is negligible.

ETIOLOGY OF THE DISEASE.

Previous Work:

H. C. Delacroix (1905) states, "In September, 1904, several attempts at infection were made. The inoculation made on the leaves without wound, simply by placing the spores on them, did not succeed except when the surrounding air was thoroughly saturated, and under these circumstances, I was successful with but one infection. The eight which I tried to inoculate by a very slight scratch on the leaf gave better luck, the infection resulting under these conditions were five out of eight attempts." The only other recorded attempt to determine the pathogenicity of this fungus was performed by H. T. Güssow (1908). "A water infusion of diseased leaf tissue sprayed on a healthy plant." "The whole foliage, stem and young fruits were covered with spots." From the meager experiments of Delacroix the impression that the organism is merely a wound parasite would be justified while the experiment of Güssow is inconclusive.

Formal Proof of Causation:

(a) Constant association of organism with the disease:

Suspected "leaf-spot" material was collected from Earliana tomatoes in the Botanical greenhouse. The typical spots were examined. Pycnidia were found containing long, filiform, septate spores. Comparing this material with descriptions of the fungus from various sources, the fungus was diagnosed as *Septoria lycopersici* Speg.

(b) Pure culture:

A pure culture was obtained by the ordinary dilution methods. The material was carefully washed under a steady, fine stream of sterile distilled water. Pycnidia were crushed on a slide and plates were poured. These were incubated; later single colonies were transferred to nutrient agar. Several days later, these were examined; pycnidia were found in abundance, but no spores were present. Portions of mycelium were transferred to nutrient glucose agar, potato agar, and tomato agar. In eight days pycnidia were found containing typical, long, filiform spores. Subcultures on the same media were made and spores produced in such

quantities that the white, glistening mass exuding from the pycnidia was easily perceptible to the unaided eye. This was very fortunate as spores could be obtained free from the mycelium and the other substratum. (See Plate 4).

(c) Production of the disease on healthy plants by inoculation from culture.

A mass of exuding spores from pure culture was shaken up in sterile, distilled water. In the first attempt to produce the disease, thirty-two plants were used.[1] Sixteen plants selected as checks, were sprayed with sterile, distilled water from a DeVilbiss atomizer No. 2. The spore suspension was sprayed upon the other sixteen plants in the same manner, using a similar atomizer. These were all kept under bell jars.

The result was positive but spots on the inoculated plants were very few per leaflet and small. One check plant also showed infection. Many leaflets showed no spots at all, and others showed no more than two. Considering the number of spores applied, and comparing the results with the Van Tieghem cell in which 30% of the spores had germinated, the results were not highly satisfactory.

The following experiment was performed. By means of a platinum loop a drop of spore suspension was placed upon a single leaflet; a very small piece of "White Rose" cotton was quickly spread over it. Twenty-five such inoculations were made. The plants were placed in a moist chamber, in which there was an arrangement to produce a fine mist when desired.[2] In five days every leaflet inoculated showed typical, water-soaked, diseased areas. The uninoculated leaflets were free from disease. Pycnidia were evident after ten days and exudation of white spore masses thirteen days after inoculation. The advantages of this method of inoculation were: the spores were protected by the cotton since the moisture collecting on the leaves did not remove them; the cotton indicated exactly where the inoculation was made; every uninoculated leaflet was a check on the inoculated leaflet.

(d) Reisolation and (e) Reinoculation:

Plates were poured from the exuding spore masses from the pycnidia and reisolations were made to tomato agar, nutrient agar, and potato agar. The identity of the recovered organism was determined beyond question. The cultures so obtained were used successfully in subsequent inoculation experiments.

Infection Phenomena:

The Period of Incubation:

Numerous experiments gave entirely concordant results as to the time necessary after inoculation for noticeable lesions to appear. In the extensive experiment reported on page 33 the daily observation required in this experiment gave abundant opportunity for exact record. The first signs of the spots appeared five days after inoculation. In experiments where inoculations were made on media of various kinds and these media incubated among the inoculated plants to insure more or less similarity of conditions, it is noteworthy to observe that the colony on tomato agar was approximately the same size as the spot on the leaf.

[1]This work was done in a greenhouse where the temperature varied from 18° C. to 25° C, and the ordinary moist greenhouse conditions prevailed.

[2]A fine jet of water from the tap was allowed to strike the top of a large galvanized iron box.

This set of phenomena indicated that the initial attack of the fungus produces little effect on the host, and it is only after several days that the fungus, probably by its by-products kills the tissues. While no work has been done with the by-products of this fungus, it may be mentioned that coincident with the killing of the host tissue occurs the darkening of the tomato agar. Experimentation with such products would undoubtedly yield results, opening up a practically untouched field of Plant Pathology.

The Subsequent Development:

On the sixth day submerged pycnidia could be seen in the inoculated leaf. The record with media was similar. On the eighth day the pycnidia were erumpent. Sections at this time showed that spore formation was beginning. On the thirteenth day exudation of spores occurred and this was taken as an indication of maturity of the spores. The record with media paralleled this with the exception that the spore exudations were more extensive in the moist test tube.

Infection and Moisture:

In the experiments so far reported in testing the pathogenicity of the fungus the inoculated plants were left in the moist chamber—sometimes in a chamber filled with a fine spray—for a comparatively long period. Experiments were later inaugurated to find the approximate number of hours requisite in the moist chamber to insure infection.

Previous experiments had shown that 100% infection followed the placing of viable spores on the under side of leaves. The flecks of cotton were found valuable as a marker and as a device for preventing the washing of the spores from leaf to leaf. Five leaflets each of ten strong vigorous plants about 7-14 inches tall were inoculated with spores from the stock pure culture. Nine[3] of these plants were put in the Wardian case which was provided with a jet of water which struck the top and broke into a fine spray. A plant was taken out at intervals of 6, 12, 24, 36, 48, 54, 60, and 72 hours after inoculation. Each plant was immediately dried by the breeze from an electric fan. After an interval of 15 minutes these plants were placed on a shelf in the window of the laboratory. After five days all plants showed typical spots on the inoculated leaves. The other leaves were healthy. That plant which had not been put into the moist chamber showed as typical a case of infection as the others. This experiment is in striking variance to the usual experience with plant pathogenes, since it is commonly stated that the host must be wet for a number of hours at least in order for infection to occur.

On account of this contradiction the experiment was immediately repeated with exactly similar results. The technique of the two experiments was alike with the exception that in the second experiment the plants were kept in the blast of the fan, following removal from the moist chamber, until infection was evident. No variation from the normal time was observed.

An experiment was then performed in which six small plants fresh from the greenhouse were inoculated respectively on the upper and lower leaf surfaces with a loop of spore suspension. The atmosphere in the greenhouse was such that the plants were in a condition of incipient wilting and the water drop disappeared at once. (August, 1914). These

[3]One plant was not put into the moist chamber.

plants were then set in the direct sunlight on a shelf in the window. Five of these plants showed typical infection.

This experiment was repeated using for inoculation the spore mass without making a suspension in water. With a platinum needle a minute portion of this spore mass exuding from a pycnidium was transferred to the leaflets of five plants. The results were similar. All plants were infected after five days. This experiment was performed because it was thought that the drop of spore suspension evaporating, drew the spores into the substomatal chamber. The results indicate that this hypothesis is untenable.

The interpretation of this experiment is rather difficult. There can be no doubt that an extremely small amount of water is necessary to enable the spores to germinate and enter the tissues. This water may be the small amount held upon the leaves, but this is difficult to credit in view of the experiment just reported. The moist air of the substomatal chamber may be concerned. The mucilaginous spore wall undoubtedly clings tenaciously to a small amount of imbibed water. A fact to be taken into consideration in the interpretation of these results is that the spores do not germinate in less than 24 hours in a Van Tieghem cell or on a moist slide. Combining this with these experiments, the results appear most striking. It is possible that the temperature factor is important in this phenomenon.[4] It is evident that if the spore reaches the leaf, regardless of the agent involved, the spore will cause infection, providing the temperature is favorable for the development of the mycelium. It may be that in the case of other fungi as well, current ideas on the necessity of water films and dew for long periods in order to produce infection may be based wholly upon inference.

PHYSIOLOGICAL AND ECOLOGICAL RELATIONS OF THE CAUSAL ORGANISM.

Germination:

To observe the spore germination and subsequent colony formation in various media, the following method was employed: Petri dishes containing Van Tieghem cells were arranged according to Duggar's (1910, p. 59) method. Blotting paper was perforated so that small glass rings would be held in place while being sterilized. (Plate 3, Fig. 1). The media used were cornmeal agar (see appendix), potato glucose agar, nutrient broth, nutrient glucose agar, tomato leaf agar, and distilled water. Solid media, from which hanging drops were made, was melted and cooled to approximately 42° C. An exuding mass of spores was shaken up in each tube. Each Petri dish as described above contained seven Van Tieghem cells. A different medium was used in each Petri dish. Each hanging drop of the medium contained 4-6 spores.

Thus it was possible to make germination studies and observe development (under the microscope) until the organism had attained macroscopic size. The exact time elapsing before germination was difficult to determine, due to the manner of development of the spores. The first signs are manifested in a pronounced swelling of the spore to almost twice the original diameter. Regular, well-defined vacuoles appeared surrounded by dense protoplasm, which made the spore look

[4]These experiments were performed from August 15 to 31.

like a chain. This condition appeared in all the media at the end of twenty-four hours. At the end of 36 hours the end cells of the spores had grown somewhat irregular, slightly tapering, and somewhat narrower than the middle cells. The elongation of both ends of the spore preceding the formation of definite germ tubes was of general occurrence (Plate 9, Fig. 3). Exceptions to this were seen when the number of spores in the hanging drops was increased so that the spores touched.

After 48 hours, germ tubes were formed varying from 1 to 4 for each spore. The germ tube may grow from any cell. (Plate 9, Figs. 1 and 2).

One may consider the germination of the spores as beginning either with the formation of the definite germ tube or with the growth from the terminal cells. In many spores instead of developing germ tubes immediately, the terminal cells elongate and then branch. The other cells in the spore may or may not send out germ tubes. For the first four days, the germination phenomena and initial colony formation are much alike in various media (Plate 9, Figs. 4 and 5). With the subsequent growth, differentiation takes place.

In the Van Tieghem rings with nutrient glucose agar and potato glucose agar a very luxuriant growth took place. The four-day-old colonies were readily visible to the unaided eye. The hyphae were richly branched, with prominent oil drops, and were very striking because of their irregular contour. (Plate 9, Fig. 7, Plate 8, Fig. 5). After six days, the medium became brown, and after eleven days, the mat-like stroma was so thick that observation with a microscope was impossible.

In distilled water the ring cultures made but slight growth other than germination and no sporulation was seen.

Cornmeal agar and tomato-leaf agar gave a moderate growth in marked contrast to the media rich in sugars. After four days, secondary spores—long filiform structures arising from small papillae on the mycelium—were found in tomato-leaf agar and two days later in the cornmeal agar. After eleven days, some of the threads of mycelium became dense brown. (Plate 9, Fig. 6). This condition was found in both cornmeal and tomato-leaf agar. (Plate 8, Fig. 4). Pycnidium formation was noticeable after the eleventh day. (Plate 9, Fig. 8).

In nutrient broth cultures, a diffuse rapid growth took place. The hyphae were narrow and regular, and were not profusely branched. On the sixth day, secondary spores were found. Five days after formation it was found that these secondary spores had germinated forming new colonies. (Plate 8, Fig. 3). Others produced secondary spores immediately after elongation (Plate 8, Fig. 2). After eleven days pycnidium formation was evident throughout the culture.

The observations may be summarized as follows:

1. Spore structures, evidently secondary spores, are formed abundantly in various media. These may germinate and form new colonies. This contradicts Delacroix (1900) who states, "The stylospores germinate easily in water and nutritive liquids by the production of a septate, branching filament *without* the formation of secondary spores."[5]

2. The presence of a distinct type of mycelium; whether this mycelium is a special stroma or pycnidial mycelium has not been definitely de-

[5]As the nutritive liquids used were not mentioned, repetition of Delacroix's experiment was not possible.

termined. It is believed that these hyphae (as a base) become associated with the hyaline hyphae to form the pycnidial wall. It might be mentioned that all media become strongly browned immediately after the pycnidia begin to form. The source and nature of this browning must remain for future investigation.

Growth on Media:

Macroscopic Cultural Observation:

The following media were used: Cornmeal agar, nutrient glucose agar, prune juice agar, tomato agar, nutrient saccharose agar, nutrient broth, bean pods, potato plugs, tomato leaves, tomato stems, cornmeal and rice.

Spores from pure culture were inoculated into three tubes of double distilled water so that a microscopic examination gave about 36 spores in a loop. One loop of spore suspension was introduced in each tube of media. This experiment was carried on in triplicate, one tube being sealed with sealing wax,[6] three tubes of each medium making up one series. Five of these series were arranged. These were allowed to grow at room temperature for 48 hours; Series 1 was placed in the greenhouse under the plants that were severely affected with the disease (Approximately room temperature); Series 2 in the ice box, (Approximately 12°); Series 3 in 37½°; Series 4 (room temperature, about 21° C.) on shelf in the window of the laboratory; Series 5 (room temperature) diffused light.

In the accompanying table the growth on different media for 3, 6, 8, 10, 12 and 18 days respectively is described for Series 1. Series 3 made no growth whatever at 37 ½° and after ten days was placed at room temperature but did not revive. All the other series were so nearly identical that it is needless to differentiate between them. The organism made no growth on bean pods. Tomato agar and cornmeal agar were found to be the best media. In the former the growth simulated more closely than in any other medium, the growth on the leaf. The excessive stroma production found in other media was absent. The pycnidia were separate and distinct, the development almost identical as to the time necessary for pycnidia production after inoculation. The tomato agar seemed to inhibit the growth of molds and bacteria when plates were poured from field material. The use of the autoclaved tomato leaf was also very satisfactory, although here no definite spots or colonies were formed, the growth being diffuse.

The following table gives the result of this experiment:

[6]Reported on page 23.

TABLE 1. GROWTH OF *SEPTORIA LYCOPERSICI* ON VARIOUS MEDIA:

Media.	3 Days.	6 Days.	8 Days.	10 Days.	12 Days.	18 Days.
Tomato agar	Colonies slightly larger than after 48 hrs.; mycelium scanty, medium beginning to darken; indefinite brownish spots in colonies.	Increased growth; colony 3 mm. diam. distinct black pycnidia submerged 4-7 per colony, separate.	Pycnidia larger; mycelium slightly increased; more pycnidia forming. Darkening of medium mycelium submerged.	No stroma, pycnidia glistening, erumpent, mycelium no increase.	White spore masses exuding from pycnidia perceptible to naked eye. Spore masses as large as pycnidium.	Spore masses increase in size. Growth stopped.
Corn-meal agar	Colonies no larger, but turning brown, medium beginning to darken. Mycelium both submerged and aerial.	Mycelium slightly increased, brownish green, slight evidence of pycnidial formation.	Pycnidia distinct, submerged; separate, mycelium slightly increased.	Pycnidia larger, still submerged. Mycelium scanty and very dark.	Pycnidia slightly erumpent, glistening black, all mycelium darkened, colonies distinct.	Spores exuding. Grayish white masses with pycnidia separating from the colony sitting on the surface.
Nutrient Glucose agar	Darkening of medium. Abundant mycelium, spreading, dark except on edge of colony.	Growth raised, spreading; mycelium below surface scanty. Pycnidia formation very abundant, contiguous; colonies coalescing.	Pycnidia erumpent, black mycelium only seen on edge of colony. Entire growth made up of pycnidia.	Pycnidia erumpent, grayish black, surrounded by aerial mycelium. No increase in colony growth.	Several exuding spore masses. Mycelium grayish dirty white.	Spores exuding, grayish white masses abundant. Colonies erumpent, separate. Pycnidia contiguous.
Prune Juice agar	Abundant mycelium, slightly spreading, aerial, white.	Medium darkening, mycelial stroma becoming erumpent, mycelium slightly spreading in definite dark spots.	Pycnidia formation submerged in stroma, contiguous, stroma very erumpent, not spreading.	Mycelium darkened, stroma formation. Evidence of pycnidial formation.	Black erumpent pycnidia. No mycelium in evidence.	Spores exuding, white masses from a few pycnidia.
Tomato Stem	No signs of growth.	Colonies with white mycelium in evidence at different points on stem.	2 colonies on one of 3 tubes, other 2 contaminated. Slight darkening. No evidence of pycnidia.	Pycnidia forming, mycelium spreading, aerial and submerged.	Pycnidia increased in number. Erumpent, glistening black.	Exuding spores from some pycnidia, white spore masses of pycnidia.
Tomato Leaf	No signs of growth.	Slight aerial mycelium, white.	No darkening of tissue. Small black specks at point of inoculation.	Increased growth; mycelium, aerial, pinkish.	Evidence of pycnidia on edge of colonies. Medium black on which colonies are growing.	Pycnidia erumpent, sparse, mycelium scanty. No signs of exudation.
Rice	No signs of growth.	Slight darkening of medium with evidence of mycelium on edge.	Medium black, colonies slightly increased. No signs of pycnidial formation.	Pycnidial formation contiguous. Mycelium in slight evidence, pycnidia covering entire colony.	Pycnidia erumpent, greenish yellow, spreading mycelium.	Stroma with embedded mycelium, white aerial mycelium. Exuding spores pinkish masses, long and slimy exudation.

Corn-meal.....	No signs of growth.	Slight darkening of medium. Mycelium spreading, flat submerged and aerial.	Increased darkening, spreading. Signs of pycnidial formation, mycelium yellowish.	Slight evidence of pycnidia formation in black stroma.	Pycnidia formation submerged, black, dull, covered with a black mycelium.	Pycnidia clearly distinguished spore formation. No exudation.
Nut. Sacc. agar.....	Darkening of medium, abundant mycelium, not spreading, black grayish white mycelium on edge of colony.	Growth raised, mycelium superficial. Pycnidial formation becoming erumpent. Colonies coalescing.	Pycnidia erumpent, colonies erumpent, pycnidia covering entire colony growth except the edge which is dirty gray mycelium.	Increased growth spore formation. Very erumpent colonies. Medium darkened.	Spore exudation. No spore masses, but exudation spreading.	Grayish mycelium growing above colonies.
Nut. Broth.....	White mycelial colonies 6 mm. diameter.	Increased size.	No evidence of pycnidial formation.	Those colonies that were attached to side of test tube darkening, evidences of pycnidia formation.	Erumpent pycnidia, 3-7 on colony.	Exuding spores, white spore masses.

Relation to Various Factors:

Desiccation:

Exuding spore masses from a pure culture, in which spore masses had just appeared were transferred to 15 sterile cover slips in a Petri dish. A spore suspension was made from spores from the same culture and a drop of it was placed on each of 15 other sterile cover glasses. These were kept in a sterile Petri dish when dry. After varying lengths of time (see table) these slips were lifted with a sterile forceps and planted in tubes to tomato agar. The results were as follows: (+) indicating growth; (—) no growth.

TABLE 2.—EFFECT OF DESICCATION: TEST WITH SPORE MASSES AND SPORE SUSPENSION.

Started 9-11-14. Spore Masses.	Started 9-14-14. Spore Suspension.
9-11-14 9 P. M. +	9-14-14 10 A. M. +
9-12-14 9 P. M. +	9-15-14 10 A. M. +
9-14-14 10 A. M. +	9-16-14 10 A. M. + (?)
9-15-14 10 A. M. +	9-17-14 10 A. M. +
9-16-14 10 A. M. —	9-18-14 10 A. M. —
9-17-14 10 A. M. —	9-19-14 10 A. M. —
9-18-14 10 A. M. —	
9-19-14 10 A. M. —	

This experiment determined the resistance to drying as well as the lack of influence of the matrix upon this resistance. This experiment shows that the spore, whether in mass in the mucilaginous matrix or isolated will not resist drying more than four days. The practical application of this experiment is obvious.

Heat:

As was indicated (Page 17) an entire set of cultures on various media were incubated at 37½° forty-eight hours after inoculation at room temperature (23°). These failed to develop. Furthermore, after taking these out, fifteen days later, and allowing them to remain at room temperature for four weeks, no sign of growth was evident.

This was verified by inoculating 10 tubes of tomato agar; five were placed in 37½° C. incubator, five were retained at room temperature (about 23° C.). The tubes which were incubated at 37½° C. failed to develop, while the check tubes developed normally. The tubes were removed after 5 days and kept at room temperature but did not revive after being kept one month at 23°.

Observations in the field and of material sent in from various sources, indicated a peculiar range in the size of spots. Pycnidia were present but the actual spot formed varied greatly. Some leaves would show numerous small spots separate and distinct from one another, covering the entire leaf. On some leaflets the disease was manifested as one large spot. These variations might be dismissed as due to weather conditions. But, if it is possible under ordinary weather conditions to find spots of pin-head size, may it not be that there are some weather conditions which entirely inhibit the development of the organism? That this hypothesis is tenable has been demonstrated by experimental evidence. Inoculated tubes of cornmeal agar were allowed to develop for 48 hours and were them placed in an incubator at 29°. These did not grow further in the incubator. However, when taken out to room temperature these re-

vived and grew normally. In this experiment two tubes were taken out each consecutive day after being placed in the incubator. When comparing all the tubes after ten days, the checks which were not put into the incubator at all, showed the greatest development, while the other tubes indicated a gradual lesser development respectively until finally the tubes taken out on the day of observation showed no development. All the tubes grew normally at room temperature maintaining a difference in growth as indicated by inhibition for 24 hours. That this is important practically, is obvious. It opens up a line of investigation which might make it possible to know what kind of weather is favorable to the fungus not from observation and hearsay but from actual laboratory tests. The lack of apparatus made it impossible to make these investigations more extensive.

However, to determine whether this would be verified on the living plant, the following experiment was performed. Two tomato plants of the same age and size were inoculated in the following manner. A spore suspension was made from a pure culture and one loop of this suspension was used for each inoculation. Twenty inoculations were made on each plant, ten on the lower surface of the leaf and ten on the upper surface. One plant was placed in the cool part of the greenhouse and the other was placed in the warmest part of the greenhouse. The first plant was placed at an average temperature of 23° C., and an average humidity of 63%. (These averages obtained from Lambrecht polymeter readings made several times a day). The second plant was placed so that the average temperature was 28° C. while the average humidity was 55%. All inoculations were successful. The plant under the cooler conditions showed difference in the lesions produced depending on whether upper or lower surfaces were inoculated. The inoculation on the upper surface gave spots 2-3 mm. in diameter, while those on the lower surfaces gave larger dead areas, 4-6 mm. With the plant at the high temperature, the spots produced by inoculations on the lower surface closely resembled those of the upper surface of plant 1, while the spots resulting from inoculations on the upper surfaces of the leaves were mere points. Eight infections of the ten were less than 1 mm. in diameter and the other two were but slightly larger.

This experiment indicates the difference in results obtained with upper and lower surface inoculations. There is also shown the effect of high temperature in inhibiting this organism while in the plant. Incidentally, this experiment indicates that a saturated atmosphere is not essential for infection to take place. The small loop of spore suspension with which the inoculation was made, disappeared almost at once when placed upon the plant. (See page 14).

Thermal Death Point of Spores:

This was determined by the method advocated by Novy (1899, p. 515) for use with bacteria. A spore suspension in 100 cc. of sterile distilled water was made using spores from a pure culture containing exuding masses 2 or 3 days old. These spores were placed in a shallow dish and then allowed to rise 5 or 8 cm. in a thin-walled capillary tube made by drawing out a sterile glass tube of about 4 mm. diameter. After the suspension had been drawn to the requisite height in the tube, the height can be determined by the slant at which the capillary tube is held, the

end which dipped in the suspension was sealed in the flame of a micro-burner. Then the capillary tube was scratched with a file about half an inch beyond the liquid, broken and sealed. At one time about two dozen such spindles were prepared, and were ready for use in the experiment. The use of such small quantities of spore suspension in thin-walled tubes enables the entire mass to reach the desired temperature quickly after submersion in the water bath.

A series of water baths made of heavy cast iron were filled with water at the desired temperature. Each bath was equipped with a Bunsen burner. By varying the height of the flame and frequent observations, it was possible to maintain the temperature within the range of ½ degree. Temperatures ranging from 40° to 60° C. by gradations of 2½ degrees were used. After an exposure of ten minutes the spindles were removed and dropped into cold water. A nick was filed near one end, then the tubes were rinsed in $HgCl_2$, followed by two changes of sterile water. One tip was broken off with flamed forceps. By gently heating the unbroken tip the contents of the tube were quickly forced out into the test tubes of media. The results were as follows:

TABLE 3.—THERMAL DEATH POINT OF SPORES: TEST WITH CAPILLARY TUBES

40° +	52½ −
42½ +	55 −
45 +	57½ −
47½ +	60 −
50 + (?)	

It may be concluded that the spores will not grow after being subjected to 52½ degrees for ten minutes. However, it is worthy of note that the number of spores which germinated and grew in tubes 40°—42½—45, were much more than that of 47½° and 50. At 50 only 6 colonies grew; at 47½, 15 colonies, while at 40—42½—45, the growth covered the entire surface of the medium and appeared exactly similar to check tubes. This resistance of certain spores raises the question whether these spores are especially heat resistant due to protoplasmic properties and whether the growing of these would give a heat resistant strain. This unfortunately could not be followed to a conclusion.

Relation to Light:

The Influence of Sunlight:

Three plates of cornmeal agar were poured using exuding spores from a pure culture. Two plates were exposed to the sun for three hours. After three days colonies were in evidence, with no perceptible difference in growth of exposed plates and check. Five inoculations were made on young tomato plants and exposed to the sun from 11:00 A. M. to 2:00 P. M. Five inoculations were made as checks. The inoculated plants developed typical infection, showing that for a period of at least three hours the sun had no effect upon the development of the organism. This verified the results obtained by exposing the spores in poured plates of cornmeal agar for the same length of time.

Comparison of Growth in Light and Dark:

Fourteen tubes of nutrient glucose agar were inoculated with exuding spores from a culture upon tomato agar. Seven tubes were wrapped in black paper (such as is used to cover photographic films). These

were placed in a closed tin vessel and with the checks were placed in a dark corner of the room. After seven days, examination showed no difference excepting those in the light developed aerial mycelium. This was absent in the tubes which were covered.

It has been mentioned above (page 17) that in the experiment dealing with the growth of the organism on various media, Series 1, was placed in the greenhouse under the plants which were severally attacked by the disease. Series 2 was in the ice box. Series 4 on shelf in the laboratory window and Series 5 in laboratory but in diffused light. The results indicated that these series were indistinguishable in their development.

Relation to Oxygen:

It was mentioned also (page 17) that the experiment was carried on in triplicate, one of which was sealed. When these were examined it seemed that some tubes showed exactly similar development to the unsealed while others of the sealed tubes indicated a very scanty growth. This incongruity was explained when it was found that every "sealed" tube which appeared similar to the unsealed tubes had developed small pin holes in the sealing wax. While conclusions can hardly be drawn from such an accident it may be well to note that there was no gradation from the sealed to the unsealed; the pin holes evidently serving as efficiently for the air supply or circulation as the larger and loose cotton plugs. In the tubes which were competely sealed, slight development was evident, but no pycnidia were observed.

Dissemination:

The connection of the organism to some ascomycete is commonly postulated. This postulate receives support from analogy with other Septorias (Klebahn, 1908). Potebnia (1910, p. 167) has expressed the opinion that the viability of conidia of a Septoria after wintering indicates loss of the ascomycetous stage. This certainly finds no substantiation in other ascomycetous life cycles, and is not sufficient ground for elimination of the possibility of the possession of an ascus stage. Attempts were made to find this hypothetical ascomycetous form, but were unsuccessful. Affected leaves were buried at various depths in the ground, November, 1913.[7] A careful examination of this material in March, April, and May, 1914, failed to reveal an ascus form. Old, diseased stems from the previous year's crop were examined with negative results. Dried pure cultures on cornmeal were examined, revealing no perfect stage.

It is noteworthy that on the wintered stems and leaves a remarkable increase in the fungus thallus had occurred, forming a conspicuous stroma, in which the pycnidia were immersed, without however, the loss of identity of the pycnidial walls. Statements, without citation of experimental work, have been made that the fungus lives over on the trash, (Cook, 1913, Stuckey, 1915), and on greenhouse frames and sashes (Güssow, 1908). Spores from pycnidia found upon wintered over vines were put in Van Tieghem cells. Ten per cent of the spores showed active germination. It cannot be definitely affirmed that this is the only

[7]Two pieces of wire mesh (4 mm.) 12 inches square were used for this purpose. These were lined with a thin layer of cotton which was thinly covered with fine sand. Infected leaves and stems showing abundant pycnidia were placed between these wires. This was submerged in a sandy loam soil near the greenhouse at an angle of 30° with the surface of the soil, the top slightly projecting, exposing about 3 inches of the wires.

source of infection in the field. The possibility that *Septoria lycopersici* may have some ascomycetous stage must necessarily hold in abeyance the problem of primary infection. It is very probable that part of the infection, at least, is produced by pycnidia in the old trash. The greenhouse or hot bed in which young plants are grown is an important source of infection. Seeds are planted under glass three or four weeks before they are set out in the field. Allowing five days for germination and appearance of the cotyledon there is a period of nearly three weeks in which the fungus may spread. As has been shown the period from the time of inoculation until the time of spore formation, is on the average 13 days under favorable conditions. Thus at least one secondary infection is possible after the primary infection. This infection usually shows as a series of spots about the older spot and is doubtless due to the floating out of spores from the exudations of the first formed pycnidia. Because of the comparatively long period between inoculation and spore formation, leaves are frequently found with large blotches and comparatively few pycnidia. These blotches with only a few pycnidia might be mistaken for the lesions of *Alternaria solani*.

The diseased cotyledons soon fall off and the plants are seemingly clean but a latent colony may be present on the leaves.

Numerous instances have been reported of the appearance of the disease in greenhouses in which tomatoes have never grown before. This indicates the possibility of transferrence of the fungus on the seed. Washings from tomato seeds have been centrifuged and examined repeatedly without successful results although the spore is characteristic enough to enable recognition.

It is believed, however, that the main source of infection is the trash from a previous tomato crop. It has been repeatedly demonstrated that rotation of crops diminishes the severity of Leaf-spot. If the infection came principally from the greenhouse, this rotation could hardly be so effective.

The agencies, aside from man, by which transferrence takes place are mainly water, and wind. The matrix in which the exuding spores are embedded holds the spores upon drying. These spores can be released only when soaked and in this condition they may be washed down by dew or rain. In culture pycnidia with exuding spores have retained those masses for a period of 3 months without dislodgment or germination. After this time tomato plants were inoculated with a suspension of spores and typical infection resulted. The matrix may have more or less of an inhibitory effect on the spores. A mass of spores from a newly formed exudate was placed in hanging drops but no germination resulted. Yet this retardation of germination may be due to the effects of the spores on each other, a condition strikingly illustrated in heavily seeded plates. (Stevens and Hall, 1909).

The dew washes or floats the spores about on the leaf. This causes the secondary infection which has been described. A large number of spots on the leaf usually do not develop pycnidia before the leaf dies and shrivels up. However, this dead, dried leaf which falls to the ground, if kept at all moist, has been found to develop mature pycnidia and exude spores more profusely than upon the living plant.

The important agency in dissemination is the splashing of the rain.

The rain may carry the spores from the old, diseased leaves on the ground to the healthy leaves above, or from the diseased leaf to the leaves surrounding it. It will be recalled that in staked tomatoes the disease progresses upward beginning at the lower leaves. It is a significant fact that the spots on the lower leaves are large, indicating infection from the lower surface, while the top leaves show the characteristics of upper leaf surface inoculations.

The relation to water was demonstrated in the greenhouse. Twelve plants were grown and staked. The cotyledons of these plants were diseased. These were not removed and were allowed to fall off. The plants were watered from above twice every twenty-four hours. After two weeks all the lower leaves developed typical leaf-spot. Two weeks following, these lower leaves developed a number of secondary spots and were beginning to shrivel and dry up. The leaves above these were in about the same stage as the first leaves two weeks before. The disease spread rapidly progressing upward on the vines. When the disease had advanced about 3 feet above the soil level, 10 healthy tomato plants were transplanted between these diseased plants. Watering from above was discontinued, being replaced by careful irrigation. The plants set between the diseased plants did not develop the disease even after twenty-five days. Watering from above was now resumed and in six days the leaves of all the young plants were covered with spots.

This experiment determines the fact that the spores do not fall or float from the pycnidium as soon as produced but adhere in a mass until liberated by water. In answer to the criticism that infection did not take place because of the dryness of the leaves, it may be said that the greenhouse was always humid and furnished ideal conditions for spore germination. The experiments already reported demonstrating the independence of infection and humidity also remove any objection to this interpretation of the foregoing experiment.

The wind, as an agency in the spreading of spores, may be considered as carrying the dust which contains the spores, thus inoculating other plants. The spores germinate in a filtered soil infusion, secondary spores being produced. Viable spores have also been found in the soil about diseased plants. However, experiments performed to determine the development of the organism in the soil yielded negative results; considering that exuding spores are washed down on the soil from the diseased leaves, it is very probable that the dust must be considered as an agency for dissemination of the fungus, limited however, by the length of time the spores can stand drying and the disintegrating action of the soil.

In this connection the difference in type of infection on upper and lower leaves may be significant. The lesions on the lower leaves are of the type to indicate infection from the lower leaf surface. It is easy to conceive that the top leaves—especially when the plants are staked—are out of the zone reached by splashings of rain, but especially open to infection brought by falling dust particles.

That man is an agent in spreading disease is very evident. In cultivating the plants when they are wet, the spores are transferred to all parts of the field by the machinery coming in contact with the sticky spore masses or upon the clothing. Instances of dissemination in this manner have been observed.

MORPHOLOGY OF CAUSAL ORGANISM.

The Spore:

The spores of the Septoria stage are filiform and hyaline varying from 60-120 microns in length to 2-4 microns in thickness, divided into from 3 to 9 cells. The thickness of a spore is not uniform throughout. The shape of the ends varies from pointed to globose. (Plate 8 Figure 1).

The Mycelium:

The mycelium of this fungus is composed of frequently septate, vacuolate threads. These threads have a diameter of from 2 microns to 3 microns. The mycelium may be said to show two types—the hyaline thin-walled type, and the dark, closely-septate, heavy-walled type. The former predominates during the vegetative stages of the growth while the latter precedes the stroma and pycnidium production. The character of these types of mycelium is brought out by figures. (Pls. 7 and 8).

The Pycnidium:

A number of tubes of tomato agar, cornmeal agar, and nutrient glucose agar were inoculated with a pure culture. At the end of every 24 hours after the colony was visible to the naked eye, material from these tubes was killed in Flemming's medium fixing fluid. Material was killed on successive days until exuding spore masses were in evidence.

The following schedule was followed:

Flemming's medium	24 hours
Washed in running tap water	6 hours
15% Alcohol	15 minutes
39% Alcohol	30 minutes
50% Alcohol	1 hour
70% Alcohol	Over night
80% Alcohol	2 hours
95% Alcohol	2 hours
Absolute Alcohol	2 hours
⅓ Cedar Oil — ⅔ Absolute	1 hour
⅔ Cedar Oil — ⅓ Absolute	1 hour
Cedar Oil	1 hour
Cedar Oil + paraffin	6 hours
Paraffin	2 hours

Transferred to new paraffin (52°) and embedded after 2 hours. Some sections 5 mm. thick were made. Stains used; Flemming's Triple, Delafield's haematoxylon, Haidenhain's haematoxylon.

Continuous efforts were made to obtain results with Delafield's haematoxylon and eosin as described by Durand (1911), but with no success. Although the time which the slide was immersed in eosin was varied from 2 seconds to 5 minutes, the carbol turpentine was inadequate to remove the excess stain. Durand's method was then changed in a single particular and excellent differentiation resulted; after immersing the slide in eosin, the excess stain was washed off with absolute alcohol for a second, then was immersed in carbol turpentine and was mounted in balsam. The result was that the mycelium became a deep red and was very easily distinguished from the surrounding tissue.

As a result of a study of a culture on tomato agar after 62 to 96 hours, the following steps in pycnidium formation were observed:

The mycelium begins to anastomose and interweave, becoming browned, resembling a closely woven net, so that the fungous tissue seems to be made up of individual cells more or less rounded in shape. This small oval body increases in size while the surrounding mycelium sends out branches which augment this globose body. This enlarging continues 24-92 hours. At the end of this time, the pycnidium consists of a hollow, globose body, without an ostiole, and is 100-375 microns in diameter, with a few strands of mycelium crossing the cavity within. (Plate 5, Fig. 1).

Spore formation takes place in the same manner as described in the

formation of the secondary spores, except that no definite elongated projection that might be considered as a well differentiated conidiophore is formed. The cells that give rise to the conidia are hyaline, resembling the peridial cells and can be traced to the brown thread from which they have arisen. Spores are produced to such an extent as to burst open the peridium at its weakest point and form an exuding spore mass sometimes twice as large as the pycnidium from which it arose. Usually that part of the peridium that is exposed is entirely broken up by the mass of spores. Plate 4, Fig. 1 illustrates this observation. In the pycnidium shown, (Plate 7, Fig. 2), the weakest point of the wall must have been submerged in the agar so that the thick part of the wall exposed to the air remained intact, while the exuding mass forced itself into the agar.

However, for complete elucidation of the method of pycnidial formation of this fungus, the development and formation of the fruiting body, must necessarily be studied in the host. Eight plants were inoculated. After three days, just as soon as the infection was visible as a small water-soaked spot, material was killed in Flemming's strong medium. Material was killed every day until the exudation of the spore masses began. The formation of pycnidia and spores was found to be identical in every particular to that described for agar cultures. The development of the pycnidial wall takes place in an exactly similar manner as in artificial media. After the "balling" up of the mycelium, the hollow case grows larger and larger, after which the spores are formed. These same sections also allowed the nature of the forces acting on the pycnidium to form the ostiole to be definitely determined. Due to the relation of the pycnidium to the surrounding tissue, the mature pycnidium finally comes to possess a more or less broad ostiole as has been pointed out by previous investigations. The steps in its origin seem to be as follows:

Soon after the pycnidium begins to form, two forces are in evidence:

1. That brought about by the enlargement of the pycnidium.
2. That brought about by the shrinking of the adjoining tissues of the leaf (a condition which can be readily seen with the unaided eye). The enlargement of the pycnidium may cause a rupture of the epidermis of the host by an upward pressure or if the fruiting body be completely embedded within the leaf tissue, there is no rupture of the epidermis.

While this fungous wall (only 3-5 cells) is pushing itself outward, the surrounding tissue is beginning to shrink due to the activity of abundant mycelium in the surrounding tissue of the latter, while the epidermis which had previously remained attached to the top of the pycnidium, begins to exert a dismembering force on all sides of the pycnidium. The great excess of this tension over the strength of the wall results in the pulling apart of the pycnidium and the formation of the so-called "ostiole." (Plate 7 shows this very clearly.) Along with this, the pressure of the spore mass no doubt, contributes to the rupture by its action at the point of greatest tension. In event, however, the pycnidium is formed within the tissue so that it does not protude above the

surface of the leaf, or as in many cases only slightly, with the peridium remaining intact, the only factor entering into the forming a "broad ostiole" is this pressure of spores. Spores begin to form *after the peridium has developed* exactly as they are produced in culture (see above). As soon as one spore is formed another arises from the undifferentiated hymenial layer. In such a manner, spores are produced in great abundance until they completely fill this pycnidium. This mass of spores becoming larger and exerting a gradually increasing force upon the thin 3-5 celled wall, suddenly *breaks* through this pycnidial wall at the point of least resistance. This point of course is usually the part of the pycnidium nearest the upper or lower epidermis of the leaf. At no time has a definite pre-formed ostiole been observed in culture or in the leaf. In culture, the mass of spores finding the weak point in the pycnidium in the submerged part of the fruiting body, forces itself into the agar (Plate 7, Fig. 2) breaking off the lower half of the wall, the remains of which were easily determined. It will be recalled by those who have seen various Sphaeropsidales in culture that the presence of an ostiole can often be clearly distinguished before spore exudation. The entire pycnidial formation in this fungus is strikingly different from that in the regular ostiole-forming pycnidia. There is no doubt but that in this matter of presence or absence of ostiole we have a phenomenon which could readily be made of taxonomic significance. (Von Höhnell 1911).

The Name of the Causal Organism:

Recently Diedicke (1912) in his critical revision of the Genus *Septoria*, limited this genus to forms whose fruiting layer is enclosed in a pseudopycnidial case, which is equipped with a more or less broad opening. This work was strongly confirmed by the previously published work of Klebahn (1908) and Potebnia (1910). At this point the work of Potebnia may be cited rather fully.

In a chapter on Septoria, Phleospora, and Rhabdospora he writes, "These three genera were in general placed with the Sphaeropsidales, while in Saccardo's Sylloge (III, 577; XI, 398; XXI, 975) the position of the genus Phleospora is not determined and is placed with both the Sphaeropsidales and the Melanconiales. Those species belonging here which were studies by me show that the three genera are closely related and properly do not constitute typical Sphaeropsidales forms, because the fruiting bodies are not built as typical pycnidia. The commonly tender wall of the fruiting body consists of a network of hyphae, which lines the cavity in the leaf tissue made by the destroying activity of the fungus. Such fruiting bodies are named "Pseudopycnidia." The development on artificial substrata in the majority of species of the three genera studied is in agreement. At first the conidia-forming mycelium is produced, then there arise the "Klumpen" or "Ballen," in which Klebahn has shown for Phleospora ulmi, pycnidia (Septoria or Phleospora), and micropycnidia (Phyllosticta) are formed."

So firmly convinced is Diedicke (1912) of the potency of this distinguishing characteristic and of its taxonomic importance, that he would exclude all forms lacking this pseudopycnidium from what he considers true Septorias. This he has already done with at least two species. Diedicke would put the forms possessing Phoma-like pycnidia

into the genus Rhabdospora. Thus in this classification the limits of Rhabdospora are circumscribed, and the old distinction emphasized by Allescher (1901). The occurrence on stems as contrasted with Septorias' occurrence on leaves, is discarded. Recent work on the Sphaeropsidales has proved the highly artificial character of this distinction. (Hedgcock, 1904, Harter, 1914).

The writer is wholly in accord with revisions of old genera when such revisions are based upon comparative morphological work with adequate materials. Adequate materials must be taken to mean fresh material from the host and from cultures rather than the fragmentary, dry material from exsiccati. Hand sections from their very meagerness and uncertainty cannot be relied upon. Without, at present, going into the merits of the case, this paper records the strong difference of morphology in *Septoria lycopersici* from that found in certain other Septorias, (Potebnia, 1910; Diedicke, 1912; and others).

If the name Septoria is to be applied to forms having *pseudopycnidia*, then a new genus must be provided for the tomato organism. At present more than 25 Septorias are being investigated in a similar manner to that described in this organism, and decision is withheld pending these studies. Accordingly the old genus name is retained.

The Species Name:

Spegazzini (1882)[8] first found the fungus in Argentina, South America. The Italian investigators noted a discrepancy in the description as applied to the Italian form, for Briosi and Cavara (1889) in their description of the fungus, state: "The leaves of the tomato attacked by this fungus show very numerous roundish or oblong spots, yellowish or ash colored with a thin darker margin. Perithecia appear on the upper surface. There also are found some on the lower surface of the leaf." "The perithecia are immersed in the tissue of the leaf, and have globose form, sometimes somewhat depressed, and have a parenchymatous wall, thin and olivaceous in color, and possess a broad ostiole. The spores escape in an agglutinated mass, and are of various lengths." (On the accompanying drawing the spore measurements are given as 40-120 x 2-3 microns).

"This parasite causes great injury, and attacks, according to Passerini, not only the leaves but also the stem and fruits. Furthermore, the characters which it showed in our specimens distinguished it somewhat from those indicated by Spegazzini. It does not form, as the author says, great spots which occupy almost the whole blade, nor are the perithecia scattered and mostly hyphophyllous and lenticular or hemispheric. Whence Passerini observed justly in his recent note, 'If it is not a species entirely distinct from *Septoria lycopersici* Speg., our form would at least be a form or local variety, varying from it by external characters.'"

In view of these observations, Briosi and Cavara named the leaf-spot in their locality, *Sept. lycopersici* var. *Europaea.*

[8]Spegazzini's description as given in Saccardo III, p. 535, is as follows:
Maculis magnis saepe totum folium occupantibus, sordide fuscocinerescentibus, subindeterminatis; peritheciis sparsis saepius hypophyllis, lenticular-hemisphaericis, prominulis, atris, membranaceis, contextu parenchymatico, olivaceo; sporulis bacillari-cylindraceis, bacillari-subclavulatis majusculis, 70-110-3, 3-pluriseptatis, utrinque obtusiuscule attenuato-rotundatis, hyalinis. *Hab.* in foliis languidis *Solani Lycopersici* in hortis, Boca del Riachuelo Argentinae.

Comparing the manifestations of the disease on various specimens from different localities, it was observed that the American specimens agreed closely with the description by Briosi and Cavara. Hence, it would seem that the fungus in this country answers to the description *Sept. lycopersici* var. *Europaea.*

However, observation of the fungus under a variety of conditions shows that there is not enough significance in the minor variations in size of spot, emphasized by Passerini to warrant the establishment of the variety.

As has been shown (p. 15), the size of the spot formed depends upon the number of spores with which the leaf is inoculated as well as upon which surface the leaf is inoculated. With a pure culture[9], in experiments determining the pathogenicity of the organism, a spot involving the entire leaf was frequently found. Spores from the same pure culture produced typical round spots when the spore suspension was further diluted. Spots arising from inoculation on the upper side of the leaf are much smaller and darker than those spots produced by inoculation on the under side of the leaf. In the field, the spots were very variable in size, depending upon the weather conditions. In cooler weather larger spots were produced than in hot weather. It was observed that spots formed in the secondary infection were much larger than in the primary infection. Spots with hypophyllous pycnidia were very common, both epiphyllous and hypophyllous pycnidia being usually found in the same spot. However, as a rule, the pycnidia are on the upper side of the leaf. Microscopical examination of large numbers of prepared slides showed all graduations from lenticular to globose pycnidia.

These observations are merely mentioned at this point to indicate that the activities of the fungus, affected by environmental conditions, cause manifestations sufficiently varied so that peculiarities of the lesion, which may be only functions of the intensity of attack, can hardly be sufficient basis for the formation of a new species or variety. Such a segregation to be valid would need to be based on carefully controlled conditions of the host and fungus.

The *Exsiccati* specimens accompanying the description by Briosi and Cavara were examined and could not be distinguished from specimens from American localities.

The only part of Spegazzini's description, then, which does not coincide with the manifestations as observed in this country is that the pycnidia are "most frequently" hypophyllous. Whether this is evidence that a different species or variety exists here is a question which is yet to be settled. [10]

If there is sufficient evidence that the fungus in Argentina is distinct, then it follows that we must necessarily have a name for the form found here.

The important point that must be noted is that regardless of nomenclature, the form in Europe seems identical morphologically with that

[9]In this paper all the work was done with the progeny of a single spore isolated as described on page 12.

[10]Material has been sent to Spegazzini for comparison with his type material, and with the organism as now found in Argentina.

found in this country. This must be recognized in all discussions of experiments performed by European investigators with this fungus.

Pending a comparison of the Septoria Leaf-spot from Argentina, the specific name *"lycopersici"* is used.

RELATION OF FUNGUS TO HOST.

Morbid Anatomy:

The Spore on the Host:

In order to follow the course of infection, inoculations were made on a number of plants. The epidermis was stripped off from the inoculated leaves at intervals of 6-12-24-36-48 hours. Examination showed germinating spores; at the end of 48 hours these appeared similar to spores in culture. (See p. 15). The great number of spores lengthened and grew from both ends. This was soon followed by germ tubes arising from various points on the spore. The type of infection is stomatal. (See Plate 4, Fig. 2). The mycelium may immediately enter a stoma. The spore in making its primary terminal growth in germination may enter the stoma, or if the mycelium does not come in contact with stoma immediately, the germ tube may branch and grow over the surface until it strikes a stoma. No chemotactic action leading to the finding of the stomata seemed to be manifested.

Mycelium and Tissues:

Infection may occur on both sides of the leaf. Careful study of the two types of infection were made in the various experiments when infection was desired. It was noted that when spores were placed on the upper surface of the leaflet, infection was manifested in small, narrow spots with from one to five pycnidia; the infection being more or less confined to the midrib and veins of the leaflet. Infection produced by placing the spores on the lower side of the leaflet resulted in a broad, spreading spot, and resembled for the greater part, field infection of the lower leaflets, spots forming at all points of the leaflet. This was verified by using one standard loop of spore suspension for inoculation on the upper and lower surface of leaflet, respectively.

Haustoria:

Immediately on entering the stoma, knob-like haustoria developed (See Plate 6, Fig. 2). These may be formed at once upon entering if the mycelium touches the cell wall. By enzymic action, the haustorium merely dissolves the cell wall, but does not penetrate deeply into the cell. A gradual shrinking of the pierced cells results. The mycelium grows luxuriantly through the leaf, making its greatest growth in the spongy tissue. At the end of five days the tissue is partly disintegrated and pycnidia begin to form. The mycelium commences to ball up and enlarge.

The mycelium is not confined to the disintegrating tissue. It is found in abundance through the tissue contiguous to the spot, in most cases terminating at the tracheary cells. In this outside tissue the cell relations are similar to the cells at the point of initial infection after the mycelium had grown for four days.

The period from the fifth to the thirteenth day after inoculation is characterized by the gradual shrinking and blackening of the tissues. From the half-submerged pycnidia the spores ooze out. The white spore

masses are similar to those produced in culture. They arise in a spiral form when the opening is small. When the opening is large, they resemble those in culture. The leaves do not suffer severely from the primary infection. When two or three spots are present on a leaflet, it begins to droop and the entire leaflet turns yellow. When the spores from these spots have been washed to all parts of the leaf by the dew or rain, infection may again take place and the spots appear just as the leaf shrivels up and dries. If the spores are present in abundance the leaf will die after the first infection, if a large number of spots are formed.

This condition is usually present when the tomatoes are allowed to run over the ground. The first condition is more typical of staked tomatoes. The reduction of the photosynthetic area of the plant impoverishes it and reduces the quantity of fruit produced. The fungus attacks old and new leaves alike, but due to the incubation period of five days, a vine that was almost completely defoliated will have a small tuft of terminal leaves.

Morbid Physiology:

The causal organism in its attack on the leaves eventually produces defoliation and the effect of this is evident, not only in the size of the crop, but upon the character of the fruit produced. There is, however, a period when the leaves are spotted but not otherwise affected, and then follows a period in which the spots, while not enlarging, evidently influence the plant, causing a yellowing of the adjacent tissue.

Work by Galloway (1896) with a rust of pine seemed to indicate that the cause of the vast disturbance of the pine leaves followed by leaf cast was due to the excessive water loss which took place from the rifted epidermis. This conclusion is warranted from his readings. Moreover, the whole biology of the evergreen needle seems to indicate that it may be especially susceptible to a transpiration shock such as must result from a strongly perforated epidermis; but in the tomato disease the epidermis remains intact and the yellowing proceeds very slowly and the leaves do not manifest the characteristics of transpiration shock. Moreover, the recent work by Reed and his associates, Cooley (1913) and Crabill (1915), on Gymnosporangium raises important questions. These investigators did not find an acceleration of transpiration from diseased tissue.

The conclusions of Reed could not be used directly for the fungus under discussion due to the strikingly different habit on the host. Rusts are known to conserve the tissue of the host, at least until after fruiting, while with this fungus the host cells attacked were killed in a very few days and previous to fruiting body formation.

An experiment was conceived which would aim to determine the influence of the disease upon transpiration. Due to the ease with which the tomato plant is infected by this fungus, and also, because of the general structure of the tomato plant, the experiment was possible on a larger scale than has ever been used in similar investigations. Twenty-two tomato plants were grown under identical conditions in the Botanical greenhouse from seed of Earliana variety. The soil of the pots was sifted twice before being placed in these receptacles and was saturated but not water-logged. The pots were prepared as follows:

A number of stiff one-pound cheese cartons (upper diameter 10 cm.,

bottom 7 cm.) were arranged to serve the same office as the aluminum shells used in the plant physiological laboratory. The lower third was cut off and the top portion fastened to Petri dishes with paraffin. After the paraffin had hardened, more melted paraffin was poured into the dish and the pot containing the plant was set into this paraffin before it hardened. Holes were made in the covers for the stem and corks. (Plate 3, Fig. 2). The transpiration performance of the plants was observed for a period of five days, May 2nd to 6th, inclusive, loss of weight being made up daily. At the end of this time, the number of leaves ranged from four to seven with leaflets on each leaf varying from three to seven.

Inoculations were made by placing two loopfuls of a pure culture suspension upon the lower surface of each alternate leaflet of eleven plants. The other plants were held as checks. The checks and inoculated plants were kept in a moist chamber for a period of four days to insure strong infection.[11] This was the only interval in which no weighings were taken, otherwise every twenty-four hours from May 2nd to May 20th, weighings were made and water added to bring back the original weight except that on May 12th, ten extra c. c. were added to each plant to allow for the increase in growth.

In order to determine the increase in leaf area from the time of inoculation to the time of cessation of the experiment, the following method was employed. By means of an adjustable glass plate and a tungsten light, prints were made on contrast paper (Cyko) of each leaf on every plant. These were developed and fixed in the usual manner. This was done just before inoculation, and immediately after the last reading on May 20th. By means of a planimeter, the area of the prints at various periods was obtained.

The disease was severe enough to cause the death of approximately two-thirds of the tissue of every leaflet inoculated. The tabulated data indicate the weighings and water added during the performance period when no plants were inoculated, and the last six days when 11 of the plants were infected.[12] This gives the data as to the amount of transpiration from the time that infection was visible to the first signs of general yellowing (break down) of the leaves. It is particularly necessary to bear in mind this point since this experiment was concerned only with the spots formed by the fungus and not with the secondary yellowing of the leaf which follows infection.

TABLE 4.—TRANSPIRATION OF HEALTHY AND DISEASED PLANTS: TEST BY WEIGHING PLANTS AND MEASURING LEAF SURFACE.

	Performance record for first 5 days of experiment.			Performance records of checks and diseased for last 6 days.			
Condition of plant.	Av. area (1) in sq. meters.	Av. daily transpiration.	Transpiration per sq. m. per hour.	Av. area (2) in sq. meters.	Av. daily transpiration.	Transpiration per sq. m. per hour.	Area 2 ÷ 1.
Healthy thruout..	.00862	13.	62.8	.02376	32.67	56.93	2.47
Inoculated after first 5 days....	.00904	13.1	60.3	.02214	30.9	58.2	2.88

[11]At that time the independence of moisture and infection was not known.
[12]The detailed tables are given in Appendix, those given in the text being summaries.

The above table emphasizes the similarity of the check and inoculated plants. Starting with the same average leaf surface and same transpiration per unit of area, even after more than ten days invasion by the fungus, we find the average area and average transpiration approximately the same. The difference in growth was not great.

If we examine the individual plants we are struck by the ranges in each of these relations; but careful comparisons will show that no greater variation occurs in the inoculated plants than occurs in the check plants. Moreover, both sets, before the inoculation took place, showed a similar variability. Our experiments cannot hope to avoid errors which come under this head. The number of plants possible for manipulation is so few in comparison to the number needed to eliminate error from this source, that it can hardly be hoped that experiments of this type can ever be entirely free from this factor. The following points may be made:

The method of obtaining the area eliminated a large source of error commonly present in area determination. The method of obtaining the amount transpired per plant was accurate to at least ½ gram, if not 1-10 gram. Moreover, the amount used is an average of several readings. The amount transpired per unit of area in the unit of time is very similar in each average.

Considering that in the inoculated plants, ⅔ of each alternate leaflet was diseased, we have the striking conditions of almost identical performance.

Necessarily the interpretation of this condition affects the whole experiment. We can have the following possibilities:

(1) Healthy and dead areas transpiring alike.
(2) Dead areas allowing more evaporation.
(3) Dead areas hindering water loss.

Were the first case true, the similarity of ratios is very readily accounted for, but then the death of the leaf, and the reduction of vitality must be explained on grounds other than mere water loss.

In event of the second, the yellowing of the leaves, the leaf-cast, etc., could readily be attributed to excessive water loss. Since, however, the ratios of transpiration for the leaves are almost the same, we need to postulate a repression of transpiration from the living areas.

In case the third condition were true, we need to postulate an augmented evaporation from the unaffected portion of the leaves, and attribute this to a chemical stimulation perhaps, from the by-products of the fungus.

To decide which of these conditions really existed, the following experiment was performed, with leaflets in early stages of the disease, and with leaflets which had been diseased for some time. Leaflets showing a few well defined spots were attached by the petiole to small rubber tubes. Other parts of the leaf were cut away and the cut surfaces covered with vaseline. All manipulations of attaching leaf to the rubber tube were carried on under water. The rubber tube was then attached to a potometer set up as shown in diagram. (Figure 2). In order to have conditions of humidity and temperature constant, the work was performed in an enclosed chamber (approximately 2 feet in each dimen-

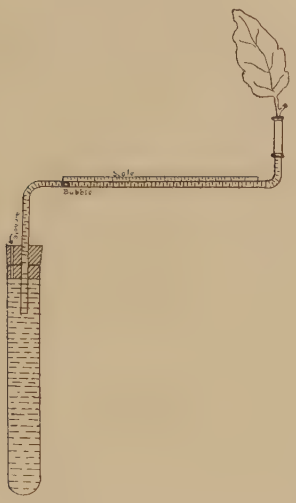

Figure 2. Diagram of potometer used to determine if diseased spots transpire.

sion). Three Lambrecht polymeters were placed in various parts of the chamber and the thermometers and hygrometers were read at each reading of water loss. The day was rather moist, and no variation occurred during the experiment. After noting the transpiration over a definite period, the diseased spots were carefully vaselined on both sides; and transpiration was noted as before. The following results were obtained:

TABLE 5.—TRANSPIRATION OF LEAFLETS IN EARLY STAGES OF DISEASE: MEASUREMENTS OF AREAS AND DISEASED SPOTS.

Condition of leaflet.	Area of 1 surface.	Number of spots on leaflet.	Diseased area 1 surface.	Per cent of leaflet diseased.
1. Leaflets green; spots pliable	1.6 sq. in. (by planimeter)	9	.054 sq. in.	3.3%
2. Leaflets green; spots pliable	2.05 sq. in.	5	.03 sq. in.	1.4%
3. Leaflets green; spots pliable	2.4 sq. in.	32	.192 sq. in.	10. %
4. Leaflets green; spots pliable	2.3 sq. in.	7	.084 sq. in.	3.6%
Average				4.8%

TABLE 6.—TRANSPIRATION OF LEAFLETS IN EARLY STAGES OF DISEASE: PERFORMANCE OF POTOMETERS.

Potometer	Movement of bubble before diseased spots were vaselined.		Movement of bubble after diseased spots were vaselined.		Rate (a).	Rate (b).	Ratio of water of movement b ÷ a.
	Time.	Spaces.	Time.	Spaces.	Before (spaces per min.)	After (spaces per min.)	
1	33 min.	9	123 min.	27	.27	.22	.81
2	83 min.	12	74 min.	41	.749	.51	.70
3	40 min.	22	103 min.	29.5	.55	.19	.35
4	41 min.	22.5	114 min.	31	.54	.27	.50
Average							.59

TABLE 7.—TRANSPIRATION OF LEAFLETS IN LATER STAGE OF DISEASE: MEASUREMENT OF AREAS AND DISEASED SPOTS.

Potometer.	Condition of leaflet.	Area of 1 surface.	Number of spots on leaflet.	Diseased area (1 surface).	Per cent of leaflet diseased.
5	Leaflet yellowed; spots dry	1.4 sq. in. (by planimeter).	16	.192	13%
6	Leaflet yellowed; spots dry	.5 sq. in.	11	.08	16%

TABLE 8.—TRANSPIRATION OF LEAFLETS IN LATER STAGES OF DISEASE: PERFORMANCE OF POTOMETERS.

Potometer.	Movement of bubble before diseased spots were vaselined.		Movement of bubble after diseased spots were vaselined.		Rate before (a) (spaces per min.)	Rate after (b) (spaces per min.)	Ratio of rates of movement (b ÷ a).
	Time.	Spaces.	Time.	Spaces.			
5	42 min.	2.5	92 min.	5.5	.06	.06	1.
6	39 min.	7	88 min.	13.5	.18	.15	.83

In the cases where the leaflets were green and spots pliable, (Tables 5 and 6), the decrease in rate of transpiration after the vaseline was applied is far greater than the mere decrease in transpiring surface would warrant. Although the spots were on an average only 4.8 per cent of the total leaf surface, the transpiration was cut down nearly one-half (.59) by vaselining the spots. This indicates that in early cases of attack, the diseased spots evaporate more than the healthy portions.

In the potometers with the yellowed leaves, (Tables 7 and 8), there

was little or no transpiration from the diseased spots, indicating that with complete desiccation and shrinkage, the spots are cut off from the water supply.

Of the three possibilities outlined on page 34, the experiment points to the second case where the diseased areas transpire more and the healthy portions of the leaflets less, but the writer is fully aware of the meagreness of his data.

IMMUNITY PHENOMENA.

Cross Inoculations:

The following plants related to the tomato were inoculated by placing a loopful of spore suspension from a pure culture on various leaflets.[15]

Solanum quinescens
Capsicum frutescens
Nicotiana tabacum (tobacco)
Solanum tuberosum (potato)
Solanum villosum

Within five days the potatoes showed definite small black spots on all the inoculated leaflets, while the check leaflets showed no infection. None of the other plants showed any spots.

The experiment was repeated with the same results. Various attempts to find pycnidia in the infected areas of the inoculated potato leaves have failed in these preliminary experiments. This is a most interesting result inasmuch as no Septoria has been described upon potato (Saccardo). It is also hard to explain the absence of such a record in view of the frequent association of tomatoes and potatoes in the field, unless the failure to produce pycnidia is a uniform condition. More extensive experiments are planned. At present, it is important to note that these spots were obtained by merely applying the spores, not injuring the tissue in any way. The importance of further investigation upon this point is obvious.

Susceptibility of Varieties:

The following varieties were obtained from D. M. Ferry & Co., Detroit, Mich., Vaughan Seed Co., Chicago, Peter Henderson & Co., New York, to whom thanks are due:

D. M. Ferry & Co.

Perfection	White Apple	June Pink
Buckeye State	Early Detroit	Golden Queen
Ponderosa	Early Michigan	Dwarf Champion
Peach	Stone	Chalk's Early Jewel
Magnus	Acme	Coreless
Beauty	Matchless	Earliana

[15]The leaflets not inoculated acted as checks.

Peter Henderson & Co.

Perfection	Chalk's Early Jewel	Stone
Tenderloin	Beauty	Livingstone's Globe
June Pink	Early Freedom	Ponderosa
Crimson Cushion	Dwarf Champion	

Vaughan Seed Co.

Red Plum	Sutton's Best of All	Golden Queen
Bonny Best	Enormous	Yellow Peach
Trophy	Lorillard	Vaughan's Model
Stone	Yellow Plum	Ground Cherry or Husk
Livingstone's Beauty	Fall Champion	Livingstone's Globe
Ponderosa	Sterling Castle	Cream City
Crimson Cushion	Pear-shaped Yellow	Red Peach
Freedom	Atlantic Prize	Chalk's Jewel
Dwarf Stone	Red Cherry	Early Michigan
Matchless	Frogmore Select	Imperial
Magnus	Honor Bright	Coreless
Acme	Comet	Buckeye State
Earliest of All	Sunrise	June Pink
Perfection	Hubert's Marvel	Early Detroit
Livingstone's Favorite	Hummer	Dwarf Champion
Pear-shaped Red	Red Currant	Dwarf Aristocrat

Ten plants of each of the varieties listed were grown. Five were inoculated after the method previously given, five were left as checks. Every inoculated plant developed the disease except the Currant tomato and the Ground Cherry. The Currant tomato, however, is by most systematists classed as a different species, and this relation to a pathogenic fungus is believed by many to be a confirmatory test of a *specific* entity. The Ground Cherry belongs to another genus (Physalis). This experiment was not intended to determine the relative effect of the disease on the plant in the field. There are a number of factors which these results do not recognize; the ability of the plant to form new leaves, the mechanical structure of the leaves, etc. This experiment, however, does point out that in the common varieties of the tomato, with this particular organism, there is no variety strikingly immune or susceptible to this disease.

It is believed in various sections of the country that the Globe is a resistant variety—this statement being often seen in horticultural papers. G. Köck (1907) classes certain varieties as resistant, less resistant, and strongly susceptible, as follows:

Resistant—Wonder of the Market, Up-to-date, Mikado, King Humbert;

Less Resistant—Magnum Bonum, Prelude, Ponderosa, Pres. Garfield and Alice Roosevelt;

Especially Susceptible—Trophy, Ficarazzi.

The varieties tested appeared neither more or less strongly affected than any other. From investigations carried on, it is found that there is no evidence of a distinction, even in the size of the spots formed among these varieties, provided the spore suspension is applied to the

lower part of the leaf with relatively the same number of spores in each inoculation. G. Köck does not mention his method in arriving at his conclusions. This, of course, precludes the possibility of comparison of results. Judging from his article he relied merely on field appearances. It is interesting to contrast the following observations by J. B. S. Norton (1914): "Practically all the varieties grown in America and Europe have been tried here the past two years, and all showed about the same amount of Septoria Blight on the foliage. Some varieties are, however, more vigorous growers and continually keep ahead of the blight by the production of new foliage."

Here as in all work on resistance and immunity in reference to plant disease, the whole matter of resistance and susceptibility is obscured by disease *Tolerance and Escape*. (Orton, 1908; Freeman, 1911). We might add to this the ability to repair or replace damaged tissue. In view of the total lack of any superiority of any one variety in the experiments reported, the writer feels that the explanation of Norton is correct and the rapidity of growth of some varieties (as contrasted with the slowness of the dwarfs) is responsible for the apparent cases of resistance to disease of certain varieties of the tomato, *Lycopersicon lycopersicon*.

RECOMMENDATIONS FOR CONTROL MEASURES.

Before control measures can be considered, we must recognize the following observations that have been dealt with above:

1. The sources of infection are at least two: the greenhouse or the hotbed and the old trash in the field.
2. Infection results from inoculation upon the upper and lower surfaces of the leaf.
3. The period from the time of inoculation to spore exudation is about 13 days.
4. Moisture is the primary agent in dissemination of the disease.
5. The exudate of spores is in the form of a mucilaginous *mass*. The spores are always transferred by some external agency.
6. It has been shown that the mycelium will not grow at 37½° F., (98° C.) and will not revive after ten days at this temperature. Since this temperature is frequently reached during the summer months, this must be taken into consideration as a natural means of checking the disease.

The most important control measures for this disease are prophylactic. Clean seedlings in clean soil, if reasonably isolated remain practically free from the disease.

In order to be sure that the seedlings do not become diseased at the start, clean soil should be used in the greenhouses devoted to seedling production. The soil should be fresh or sterilized. The seedlings should be sprayed as soon as their height above ground makes it practicable, and again before being transplanted to the field. For this a weak Bordeaux mixture (2-2-50) is advised.

Since it has been determined that wintered-over, diseased vines possess spores which are viable, the old trash must be destroyed as far as possible. Since, however, this is not practical except in greenhouses and

gardens, rotation is strongly urged. While there is no experimental evidence to demonstrate the value of rotation as a means of control, numerous instances have been noted in which rotation has been successful in controlling the disease.

After transplanting to the field, spray with 4-4-50 Bordeaux mixture every 10 days. As has been shown, the period from time of inoculation to spore exudation is at least 13 days. Allowing this leeway for differences in period of infection, it would seem that a spray so applied would give the necessary protection.

The greatest part of and the strongest infection results from inoculation on the lower surface of the leaf. Therefore, all these precautions are less effective if the plants are allowed to run at will over the ground. The spraying of the under side of the leaf is not accomplished unless the application is thorough. Failures to control the disease by spraying are doubtless due to lack of thoroughness and timeliness.

Moisture acts as a mechanical factor in disseminating the disease in two ways. (1). The dew carries the spores from the mucilaginous mass to all parts of the leaflet to form secondary infection. (2). The rain by splashing, carries the spores from the ground below to the leaves above and in a similar manner carries the spores from the diseased leaves on the ground to the healthy leaves above.

The mucilaginous matrix holds the spores until they are released by contact with some object. When the plants are dry, the spores do not float in the air or fall from the leaf but stay embedded in this matrix, which becomes hard and strongly cohesive.

Accordingly the plants should not be "worked" when wet. Growers have reported cases where cultivation began at a small infected patch and the disease was carried over the entire field, and that in less than three weeks the entire field was spotted. This is now readily explained. In greenhouse practice, irrigation should replace the ordinary use of the hose.

Spraying with 4-4-50 Bordeaux mixture in the fields where tomatoes are staked should be extremely successful. In this state spraying will not pay unless the spray reaches the lower surface of the leaves. A sprayer such as is used for potatoes, with two side nozzles set to shoot upward and if practical, with one central nozzle to spray downward for each row will, under high pressure, be most efficient in Michigan fields.

SUMMARY.

This bulletin gives the results of experiments with the Leaf-Spot disease of tomato and recommendations for its control. After a discussion of the previous work on the disease, the disease is discussed under topics:—Name, Economic Importance, and Distribution. The disease manifests itself by forming lesions on leaves, stems, calyx, and fruit.

Since previous work for the most part inferred the connection of the associated fungus and the disease, or at least was not entirely conclusive, formal proof of parasitism was undertaken. This work proved that the organism, *Septoria lycopersici* Speg., causes the disease. Studies of infection phenomena were given under the topics:—Period of Incubation, Subsequent Development, and The Relation of Infection to Moisture.

The physiological and ecological relations of the causal organism were studied under the topics:—Germination, Growth on Media, Relation to Dessication, Heat, Light, Oxygen, and Dissemination.

Morphology of the causal organism indicated that the pycnidium is a closed ball with a wall similar to that in the genus Phoma. The spores are long and needle-shaped bodies with pointed or rounded ends, usually with several septa. The mycelium is of two sorts. The morphology of this organism shows a striking difference from that found in the members of the genus Septoria studied by Potebnia, Diedicke and Klebahn. There is no proof available to show that the organism in the United States is different from that in Argentine, hence the specific name, *lycopersici*, is retained.

In considering the relation of the fungus to the host, it was found that the type of infection is stomatal. The mycelium is intercellular and possesses haustoria.

An experiment to determine the morbid physiology of the plants infected by Septoria indicated that healthy and diseased plants transpired nearly alike per unit of area. There was, however, an indication that the diseased spots transpire more than the healthy portions of the affected leaves, and that the transpiration of these healthy portions is repressed below normal. The fungus was found to infect the potato when the latter is artificially inoculated. No fruiting bodies were produced in the disease spots. No variety of tomato was found especially susceptible or immune although more than fifty were tried. Control measures, chiefly prophylactic, are recommended.

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APPENDIX.

Media Used in Cultural Work with the Fungus.

Cornmeal Agar (Shear):

Four teaspoonfuls of yellow cornmeal were added to a liter of distilled water. Digested in water bath below 60° for one hour, 1.3% agar was added and boiled for 45 minutes; water lost added; albumen of two eggs added for clearing. Filtered through absorbent cotton and tubed. Autoclaved 15 minutes 110°

Prune Juice Agar:

One hundred twenty gms. of dry prune flesh. Boiled in one liter of distilled water in steamer for two hours. Filtered through cheesecloth and 12 gms. of agar added. Boiled two hours in steamer until completely dissolved. Cleared with albumen of two eggs. Filtered through absorbent cotton. Autoclaved 15 minutes 110°.

Tomato Leaf Agar:

Green tomato leaves were pressed and 60 cc. of juice was boiled in 500 cc. of water and then 12 grams agar flour was added to juice, boiled 45 minutes and cleared with albumen of two eggs. Filtered through cotton. Autoclaved 10 min. 110°.

Nutrient Glucose Agar:

Digested 20 gms. of agar in 500 cc. of water. Dissolved in 500 cc. of water 40 gms. of glucose, 3 gms. of beef extract, 3 gms. of salt, 20 gms. of peptone. Poured into agar and steamed for 45 minutes. Cleared with albumen of two eggs. Filtered through absorbent cotton. Autoclaved 10 minutes at 110°.

Nutrient Saccharose Agar:

Digested 30 gms. of agar in 500 cc. of water. Dissolved in 500 cc. of water 40 gms. of saccharose, 3 gms. beef extract, 3 gms, salt, 20 gms. peptone. Poured into agar and steamed for 45 minutes. Cleared with albumen of two eggs. Filtered through absorbent cotton. Autoclaved 10 minutes at 110°.

Nutrient Broth:

Dissolved 3 gms. of Leibig's beef extract in a liter of water to which was added 10 gms. peptone, 5 gms. of common salt; neutralized with normal NaOH to 1° Fuller's Scale. Autoclaved 10 minutes at 110°.

Bean Pods:

Canned bean pods placed with ½ inch distilled water in test tubes. Autoclaved at 110° fifteen minutes.

Potato Plugs:

Potato plugs made with cork borer. Soaked in running water 24 hours. Placed in tubes in bottom of which a small wad of cotton soaked

in distilled water was placed. Steamed three consecutive days for 15 minutes.

Tomato Leaves:

Young leaves were cut from growing vines. These were placed in test tubes with 2 cc. distilled water. Autoclaved at 110° for 5 minutes.

Tomato Stems:

Stems were prepared in the same manner as the leaves.

Cornmeal:

A teaspoonful of cornmeal in large test tube to which was added four times volume of distilled water. Steamed 3 consecutive days for 15 minutes.

Rice:

Rice was prepared in the same manner as cornmeal.

TABLE 9.—TRANSPIRATION RECORDS OF HEALTHY PLANTS IN EXPERIMENT REPORTED ON PAGE 33: PLANIMETER READINGS MADE IN SQUARE INCHES.

Healthy throughout.

Plant.	Water loss in grams. May. 2.	3.	4.	5.	6.	Total loss in 120 hrs.	Average daily transpiration.	Area in sq. in.	Area in sq. m.	Transpiration per sq. m. per hour.
1	10	9.	8.3	3.	6.	36.5	7.3	13.95	.005510	55.17
2	26.5	27.5	23.	9.	13.	32.27	19.8	32.27	.01277	64.68
3	16.	19.	17.5	7.5	12.	72.	14.4	21.60	.00858	69.8
4	9.	9.5	10.	4.	6.	38.5	7.7	15.38	.00607	52.82
5	17.	19.	17.	7.5	13.	73.5	14.7	27.98	.01104	55.42
6	14.	16.	13.	4.	7.5	54.5	10.9	14.72	.00582	77.95
7	14.	13.5	11.	4.5	11.	54.	10.8	21.27	.008405	53.5
8	19.	16.5	13.5	2.5	12.5	64.	12.8	21.19	.00837	63.7
13	21.	21.	20.	8.	15.	85.	17.	30.22	.01195	59.25
14	33.5	30.5	24.5	12.	20.5	121.	24.2	37.67	.01490	67.8
16	18.	17.5	14.	7.5	12.5	68.5	13.7	23.67	.008365	68.25
Average							13.	21 81	.00862	62.8

Plant.	Water loss in grams. May. 15.	16.	17.	18.	19.	20.	Total loss in 144 hrs.	Average daily transpiration.	Area in sq. in.	Area in m.	Average transpiration per sq. m. per hr.	Gain in area in sq. m.	Area (2) ÷ by Area (1).
1	22.	20.5	28.5	26.	28.	28.5	153.5	25.58	52.04	.02058	37.24	.01507	3.7
2	43.	39.	47.	43.	45.	42.5	259.5	43.25	66.51	.02625	68.52	.01348	2.
3	43.5	36.5	48.5	44.	46.	43.5	262.	43.6	67.69	.02665	69.65	.01807	1.9
4	31.5	26.5	34.5	36.5	43.	43.	215.	35.83	60.29	.02380	62.7	.01773	2.9
5	35.5	28.5	37.	36.5	38.	34.5	210.	35	63.77	.02520	57.8	.01416	2.2
6	22.	19.	26.	21.5	24.5	21.5	134.5	22.42	33.55	.01325	71.04	.00743	2.2
7	30.	29.5	33.	33.5	33.5	31.5	191	31.83	67.51	.02664	49.75	.01823	3.1
8	34.5	20.5	35.	30.5	37.	32.5	190.	31.65	48.97	.01935	68.15	.01098	2.3
13	18.5	20.5	23.	22.	21.	21.	126.	21.	61.12	.02418	36.22	.01323	2.5
14	29.5	34.5	42.	32.5	35.	32.5	206.	34.33	79.92	.03128	45 7	.02638	2.3
16	32.	24.5	39.5	38.	39.	36.	309.	34.83	61.32	.02420	59.99	.01584	2.6
Average								32.67	60.24	.02376	56.93	.01546	2.47

TABLE 10.—TRANSPIRATION RECORDS OF INOCULATED PLANTS IN EXPERIMENT REPORTED ON PAGE 33: PLANIMETER READINGS MADE IN SQUARE INCHES.

Inoculated on May 7.

Plant.	Water loss in grams. May. 2.	3.	4.	5.	6.	Total loss in 120 hrs.	Average daily transpiration.	Area in sq. in.	Area in sq. m.	Transpiration per sq. m. per hour.
9	14.5	16.	15.	6.	11.5	63.	12.6	28.61	.01131	46.4
10	17.5	16.	14.5	5.5	12.5	66.	13.2	23.64	.00934	58.85
11	16.	18.5	16.	6.5	12.	69.	13.8	24.16	.00955	60.2
12	9.5	9.5	9.	3.5	7.	38.5	7.7	12.53	.004958	64.73
15	28.	26.5	20.	11.5	18.5	104.5	20.9	36.37	.01396	62.4
17	19.5	20.5	17.	8.	13.5	78.5	15.7	23.49	.00928	70.4
18	11.5	11.	9.5	3.5	6.	41.5	8.3	9.56	.00377	91.6
19	20.	20.	18.	10.5	14.5	83.	16.6	27.25	.01078	64.18
20	11.	13.5	11.5	4.	7.	47.	9.4	16.78	.00666	58.78
21	20.5	20.5	20.	20.	16.5	87.5	17.5	29.84	.0118	61.75
22	9.	9.5	8.5	3.	6.5	36.5	7.3	11.65	.0046	66.2
Average						22.88	13 1	22 88	.00904	60.3

Plant.	Water loss in grams. May. 15.	16.	17.	18.	19.	20.	Total loss in 144 hrs.	Average daily transpiration.	Area in sq. in.	Area in m.	Average transpiration per sq. m. per hr.	Gain in area in sq. m.	Area (2) ÷ by Area (1).
9	25.	23.	31.	31.	34.	31.	115	29.2	65.54	.02552	45.75	.01421	2.2
10	28.	25.5	38.5	38.5	41.	45.5	227	37.9	65.59	.02593	61.02	.01659	2.7
11	41.5	37.5	46.5	43.	46.	42.	256.5	42.7	71.24	.02815	63.25	.01860	2.9
12	26.5	21.5	29.	29.	35.5	37.	178.5	29.8	55.30	.02816	56.85	.01788	4.4
15	32.5	34.	49.5	38.5	43.	40.	237.5	39.6	97.01	.03839	43.00	.02438	2.6
17	40.	27.	42.5	39.	42.5	49.	231	38.5	70.10	.02808	57.15	.01880	2.9
18	14.	9.	15.	19.5	14.	12.	84.	14.	15.88	.00627	93.00	.00250	1.7
19	25.5	23.5	29.5	29.5	29.5	27.	162.5	27.1	69.31	.02738	41.3	.01660	2.5
20	30.	23.	29.5	30.5	35.	33.5	181.5	30.2	59.33	.02344	53.52	.01678	3.5
21	36.5	24.5	31.	29.	29.	29.	170.	28.3	59.70	.0236	50.00	.01180	2.5
22	21.5	10.	28.5	23.	27.5	24.	137.5	22.9	41.01	.01622	58.85	.01162	3.5
Average								30.9	60.82	.02214	58.2	.01310	2.88

DESCRIPTION OF PLATES.

Plate 1.
Diseased plant showing killing of lower leaves.

Plate 2.
Fig. 1. The disease as found in the field.
Fig. 2. Diseased spots X 5.

Plate 3.
Fig. 1. Van Tieghem cells with distilled water and nutrient glucose agar.
Fig. 2. Plants used in transpiration experiment. The pots are inside of cheese cartons which are cemented to Petri dishes with paraffin.

Plate 4.
Fig. 1. Pycnidium in the leaf, showing spores pushing out, thus making the ostiole. X 250.
Fig. 2. Test tube cultures on tomato agar and nutrient glucose agar showing spore exudation.
Fig. 3. Section of sub-stomatal chamber of leaf showing entrance of mycelium. X 1,000.

Plate 5.
Fig. 1. Pycnidum before arisal of spores showing mycelial strands running across the chamber. X 750.
Fig. 2. Magnified camera drawing showing nature of young pycnidial wall. X 750.

Plate 6.
Fig. 1. Mycelium in hanging drop culture showing arisal of secondary spores, and heavy-walled mycelium.
Fig. 2. Mycelium between the cells of the host, sending blunt, tubular haustoria into the cells. X 1,000.

Plate 7.
Fig. 1. Pycnidium on a tomato leaf, showing relation of spores and adjacent tissue to ostiole formation. X 800.
Fig. 2. Pycnidium from test tube culture showing spores masses pushing into agar. X 300.

Plate 8.
Fig. 1. Spore showing globose ends.
Fig. 2. Germinating spores producing secondary spores.
Fig. 3. Secondary spore germinating.
Fig. 4. Mycelium producing secondary spores (corn meal agar).
Fig. 5. Mycelium branching, no secondary spores (nutrient glucose agar).

Plate 9.
Fig. 1. Germinating spore (tomato agar).
Fig. 2, 4 and 5. Stages in colony formation.
Fig. 3. Spore germinating by elongation of both ends, (nutrient broth).
Fig. 6. Mycelium with secondary spores, and heavy brown threads (corn meal agar).
Fig. 7. Mycelium (nutrient glucose agar).
Fig. 8. Pycnidium formation (corn meal agar).

PLATE 1.

PLATE 2.

PLATE 8.

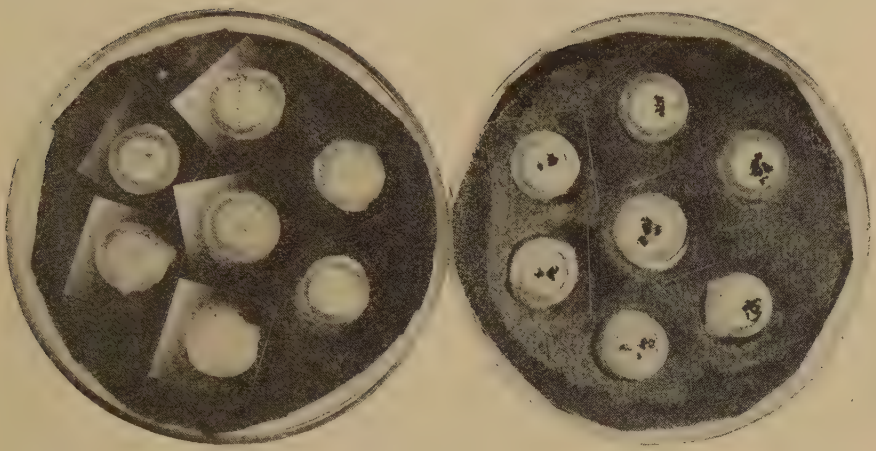
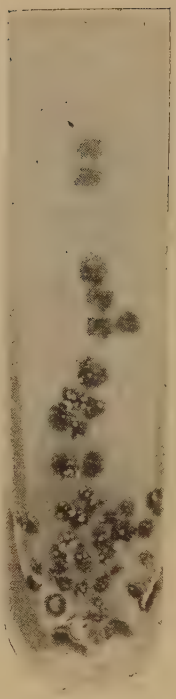
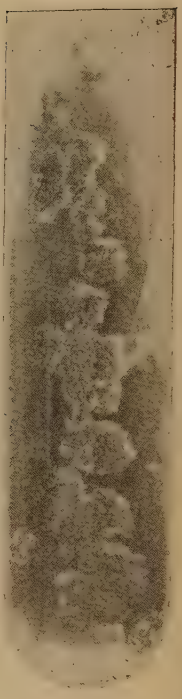
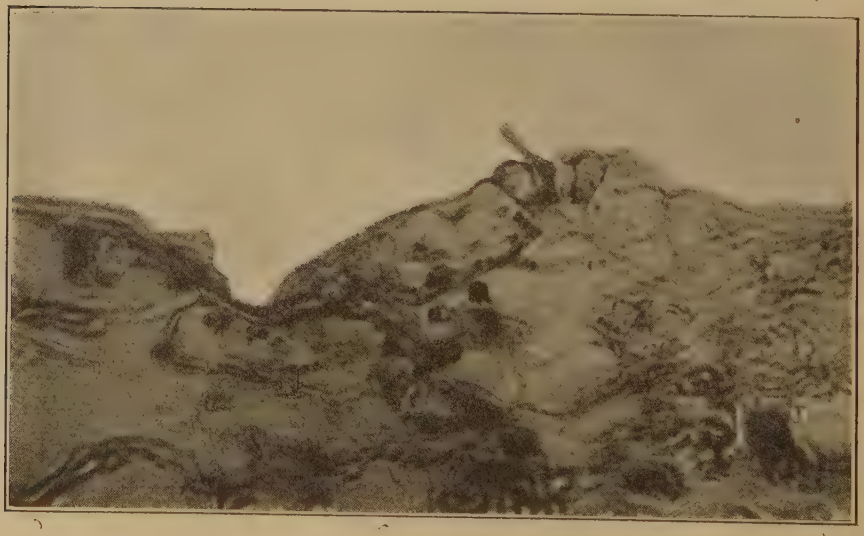


PLATE 4.







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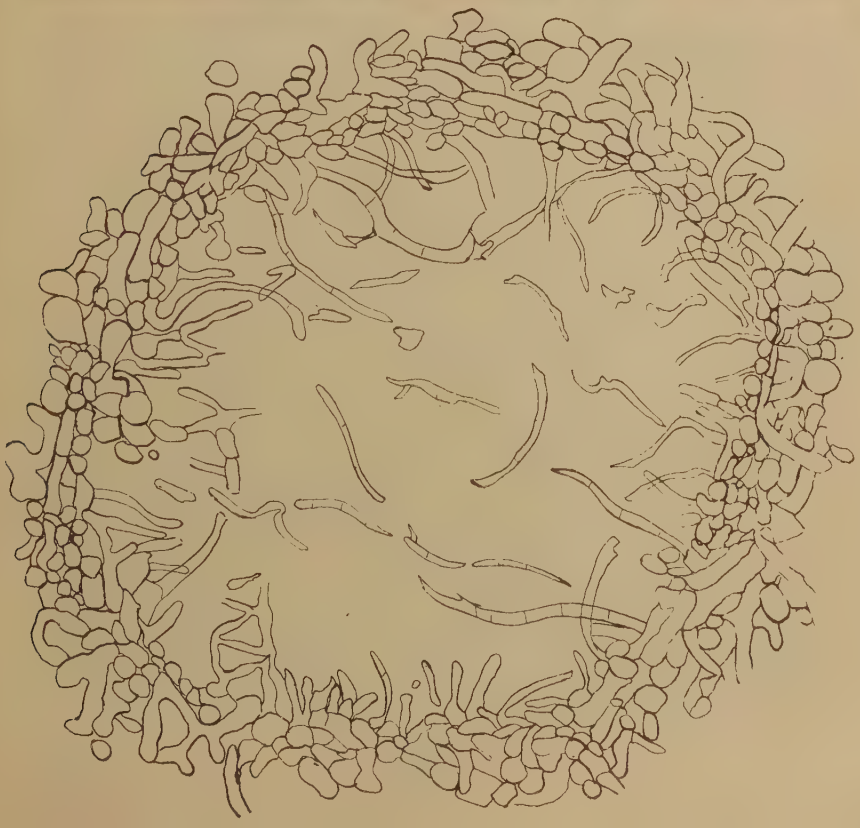

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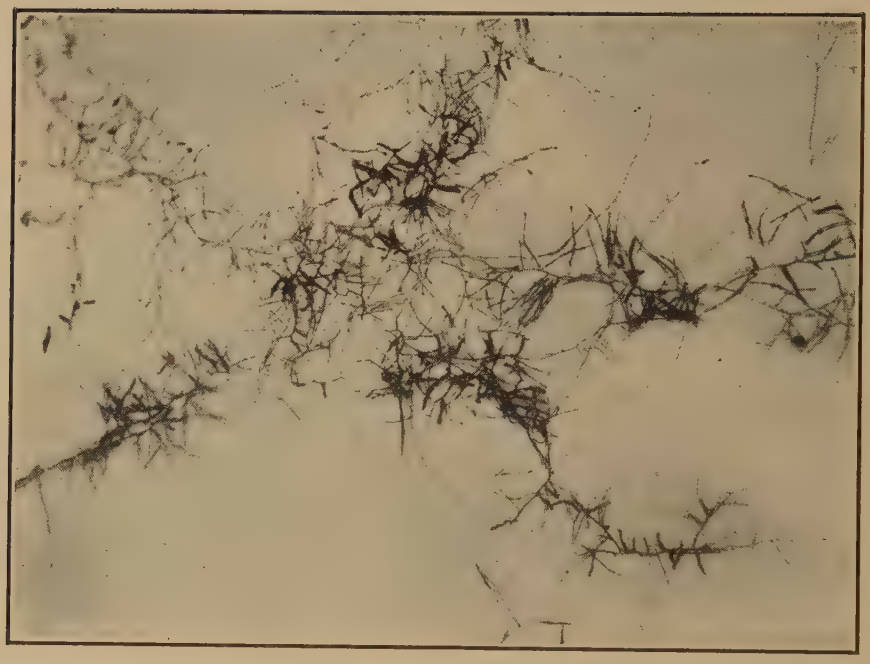

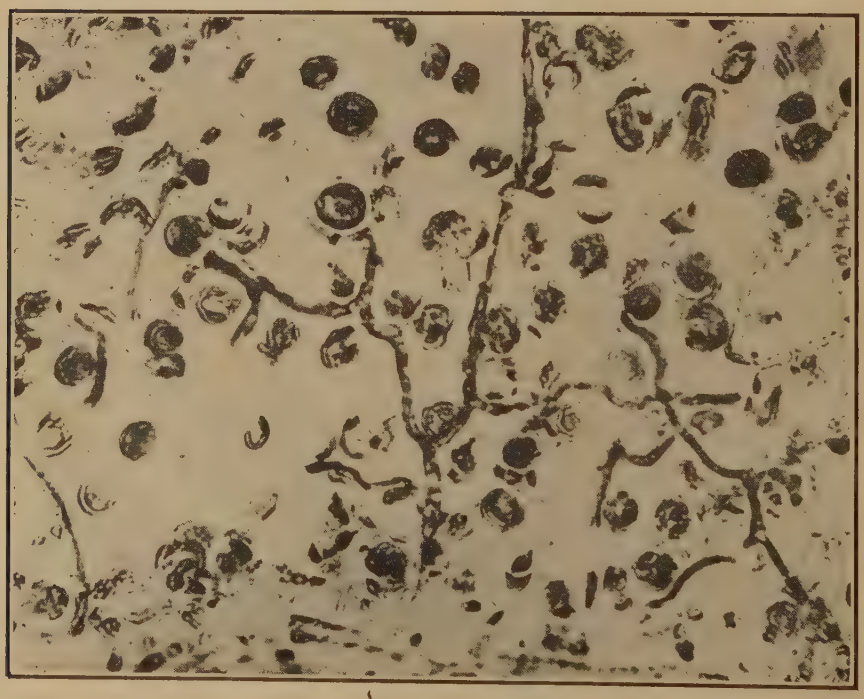

PLATE 7.

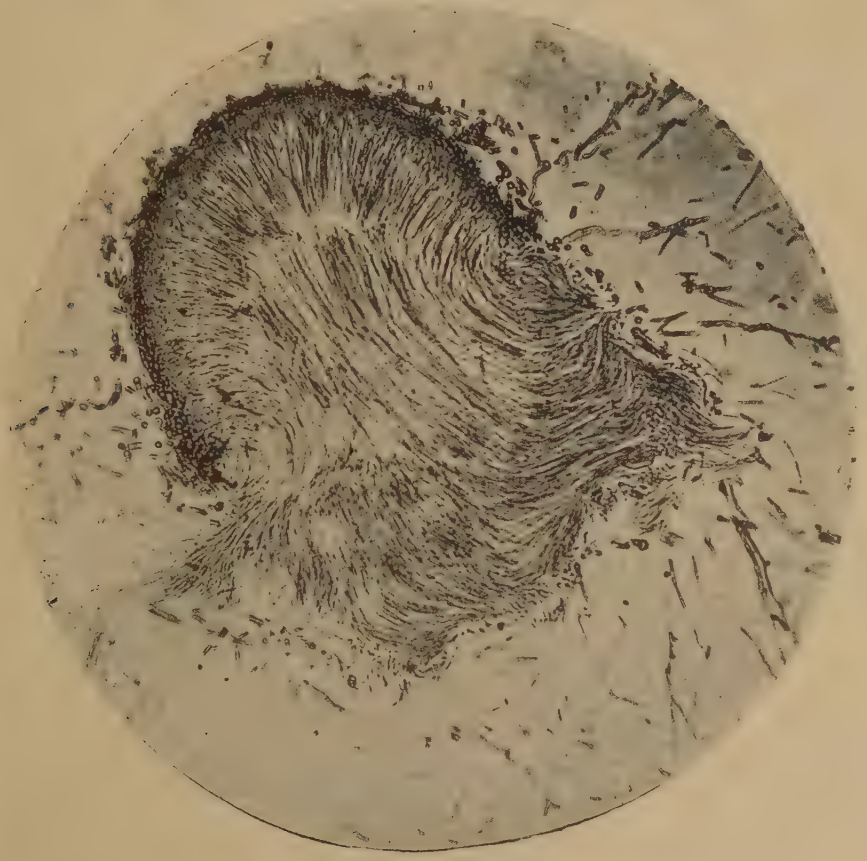

PLATE 8.

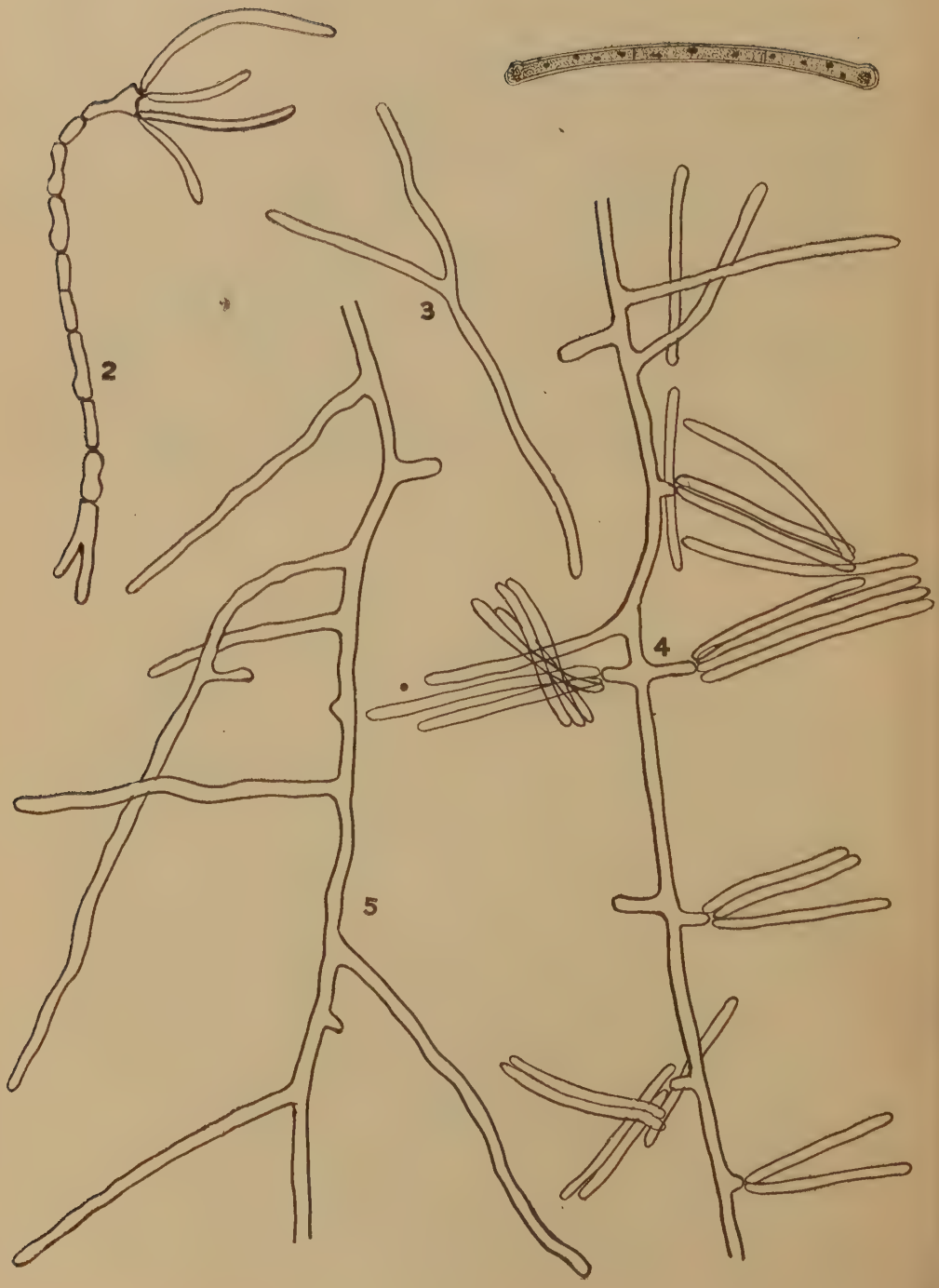

PLATE 9.